MINIREVIEW

The pyrin inflammasome: from sensing RhoA GTPases-inhibiting toxins to triggering autoinflammatory syndromes

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One sentence summary: The pyrin inflammasome: when an innate immune complex detecting bacterial toxins causes autoinflammatory syndromes.

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ABSTRACT

Numerous pathogens including \textit{Clostridium difficile} and \textit{Yersinia pestis} have evolved toxins or effectors targeting GTPases from the RhoA subfamily (RhoA/B/C) to inhibit or hijack the host cytoskeleton dynamics. The resulting impairment of RhoA GTPases activity is sensed by the host via an innate immune complex termed the pyrin inflammasome in which caspase-1 is activated. The cascade leading to activation of the pyrin inflammasome has been recently uncovered. In this review, following a brief presentation of RhoA GTPases-modulating toxins, we present the pyrin inflammasome and its regulatory mechanisms. Furthermore, we discuss how some pathogens have developed strategies to escape detection by the pyrin inflammasome. Finally, we present five monogenic autoinflammatory diseases associated with pyrin inflammasome deregulation. The molecular insights provided by the study of these diseases and the corresponding mutations on pyrin inflammasome regulation and activation are presented.

Keywords: pyrin inflammasome; RhoA; familial Mediterranean fever; toxin; PSTPIP1; autoinflammatory disease

RhoA GTPases: MOLECULAR SWITCHES CONTROLLING CYTOSKELETON DYNAMICS

Rho-family GTPases are molecular switches that control many aspects of the actin cytoskeleton dynamics (Bourne, Sanders and McCormick 1991). Twenty human Rho GTPases are included in this family (https://www.genenames.org/cgi-bin/genefamilies/set/390) with the best-characterized members being RhoA, CDC42 and Rac1. In this review, we will focus on the RhoA subfamily GTPases that have been linked to the pyrin inflammasome, namely RhoA/B/C GTPases (Xu et al. 2014). These three GTPases share over 84% identity at the protein level with most of the variations occurring in their 13–16 last amino acids. They will be hereinafter referred to as RhoA GTPases.

Depending on the nature of the bound nucleotide, RhoA GTPases cycle between an ‘ON’ (GTP-bound) and ‘OFF’ (GDP-bound)
stages that define their role as molecular switches (Fig. 1). RhoA GTPases when bound to GTP (‘ON’ state) interact with and activate specific effectors. Upon GTP hydrolysis, the affinity of GDP-bound RhoA for their effectors drops substantially leading to a loss of interaction with their downstream effectors (‘OFF’ state). While RhoA GTPases have a slow intrinsic GTPase activity, this activity is accelerated by GAPs (GTPase-activating proteins). Conversely, GEFs (guanine nucleotide exchange factors) facilitate the exchange of GDP by GTP thereby turning RhoA GTPases in their ‘ON’ state. The ‘ON-OFF’ cycle is further regulated by Rho-GDP dissociation inhibitors (GDI), which interact with GTP-bound RhoA and sequester it in its GDP-bound state. Finally, RhoA GTPases are targeted to host cell membranes thanks to a post-translational modification consisting in the covalent addition of a lipid (either a geranylgeranyl or a farnesyl) to their CAAX motif. RhoA proteins (Table 1). Importantly, several pathogens secrete both RhoA-activating and inhibiting effectors to hijack the RhoA-mediated cell processes while tightly controlling the consequences of RhoA deregulation. This is well exemplified by the study of Yersinia effectors and their impact on the pyrin inflammasome (see below) but is likely to be relevant during enterohemorrhagic Escherichia coli infections (Wong et al. 2012). Inactivation of RhoA GTPases results in multiple consequences on host cells including inhibition of cell migration, loss of epithelial/endothelial barrier integrity and cell death. On the other hand, RhoA GTPases are targeted by numerous microbial virulence factors.

**TOXINS AND BACTERIAL EFFECTORS TARGETING RhoA GTPases**

RhoA GTPases’ modulation by pathogens has been mostly studied in the context of bacterial infections (Table 1) (Aktories 2011) although eukaryotic parasites and viruses have also been reported to target these proteins (Colinet et al. 2007; Gorbunova et al. 2016; Van den Broeke, Jacob and Favoreel 2014). The convergent evolution of pathogens to target RhoA GTPases is well demonstrated by the large number of bacteria (Gram-negative and Gram-positive) that have evolved very different virulence factors targeting Rho GTPases. Exotoxins (e.g. *Clostridium difficile* toxins A and B (TcdA/B), by glucosylating RhoA GTPases, prevent their interaction with downstream effectors.

**Figure 1. Regulation of RhoA GTPases molecular switch.** RhoA GTPases cycle between ‘ON’ (GTP-bound) and ‘OFF’ (GDP-bound) stages. GTP-bound RhoA GTPases interact with and activate downstream effectors including ROCK-1/2, PKN-1/2 and FAM65A resulting in the control of multiple cellular processes, most of them including actin cytoskeleton dynamics. RhoA GTPase are targeted to host cell membranes thanks to the covalent addition of a geranylgeranyl lipid to their CAAX motif. RhoA GTPases’ activity is regulated by GAPs (GTPase-activating proteins), GEFs (guanine nucleotide exchange factors) and GDIs (Rho-GDP dissociation inhibitors). Yersinia spp. have evolved two effectors, YopE and YopT, to inhibit RhoA activity; YopE acts as a RhoA GAP while YopT cleaves the isoprenoid tail anchoring RhoA at the membrane. Clostridium difficile toxins A and B (TcdA/B), by glucosylating RhoA GTPases, prevent their interaction with downstream effectors.
hand, RhoA GTPases activation may be a mean for bacteria to promote their intracellular access or to modulate host vesicular trafficking (Lemonnier, Landraud and Lemichez 2007; Ohlson et al. 2008).

**INFLAMMASOMES: INFLAMMATORY CASPASES-ACTIVATING PLATFORMS**

Inflammasomes are innate immune platforms assembled in the cytosol in response to the sensing of pathogens or of damage-associated signals. Various inflammasome sensors have been described with three main activation modes. In the first mode, the receptor recognizes a pathogen-associated molecular pattern through direct binding (e.g. the NAIP receptor directly binding the needle protein of T3SS) (Yang et al. 2013). In the second mode, the inflammasome sensor acts as an integrated decoy and is activated upon targeting by a virulence factor (e.g. lethal factor from *Bacillus anthracis* cleaves NLRP1b leading to its activation) (Chavarria-Smith et al. 2016). In the third mode, the inflammasome sensor acts as a dynamic sensor of homeostasis (e.g. NLRP3 senses perturbation in cytosolic K⁺ concentration following membrane damage by pore-forming toxins) (Petritili et al. 2007). Pyrin belongs to this last class of sensor by sensing RhoA GTPases activity.

Engagement of an inflammasome sensor leads to assembly of a macromolecular complex, known as the inflammasome, in which caspase-1 is activated. Caspase-1 is an inflammatory caspase triggering interleukin (IL)-1β and IL-18 maturation and release, and an inflammatory form of programmed necrosis termed pyroptosis (Bergsbaken, Fink and Cookson 2009). Pyroptosis results from the caspase-1-mediated cleavage of gasdermin D (GSDMD) leading to the release of the GSDMD N-terminal polypeptide (Kagayaki et al. 2015; Shi et al. 2015). This N-terminal polypeptide acts as a eukaryotic pore-forming toxin. It is targeted to the inner leaflet of the plasma membrane where it oligomerizes and forms pores (Ding et al. 2016; Liu et al. 2016). The resulting disruption of plasma membrane integrity leads to osmotic swelling, releases inflammatory cytokines and culminates in pyroptotic cell death.

**PYRIN: A SENSOR OF RhoA GTPases ACTIVITY**

Pyrin is an inflammasome sensor that detects inhibition of RhoA subfamily GTPases activity. In contrast to other inflammasome sensors from the NLK (nucleotide-binding domain and leucine-rich repeat containing) family (Ting et al. 2008), the pyrin protein has a unique structure (Fig. 2). In addition to a PYD domain (required for interaction with ASC) in its N-terminal part, pyrin features a B-box domain followed by a coiled-coil domain and a B30.2 domain. The PYD and the B-box domains are joined by a 278-amino-acid long linker that contains two serine residues playing a key regulatory role (see below). The B-box, the

### Table 1. RhoA-targeting bacterial effectors/toxins in human pathogens.

<table>
<thead>
<tr>
<th>Activation</th>
<th>Bacteria</th>
<th>RhoA modification</th>
<th>Cellular function</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>EspM2</td>
<td>Enterohemorrhagic Escherichia coli</td>
<td>GEF</td>
<td>Adhesion onto cells</td>
<td>Arbeloa et al. (2010); Wong et al. (2012)</td>
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<tr>
<td>IpGB2</td>
<td>Shigella flexneri</td>
<td>GEF</td>
<td>Entry into polarized cells</td>
<td>Alto et al. (2006); Hachani et al. (2008)</td>
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<tr>
<td>CNF1/2</td>
<td>Escherichia coli</td>
<td>Deamidation</td>
<td>Entry into host cells</td>
<td>Flatau et al. (1997); Sugai et al. (1999)</td>
</tr>
<tr>
<td>CNFy</td>
<td>Yersinia pseudotuberculosis Bordetella spp.</td>
<td>Deamidation</td>
<td>Inflammation modulation and disruption</td>
<td>Schweer et al. (2013)</td>
</tr>
<tr>
<td>DNT</td>
<td></td>
<td>Deamidation/transglutamination</td>
<td>Dermoncrosis</td>
<td>Schmidt et al. (1999)</td>
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<tr>
<th>Inhibition</th>
<th>Bacteria</th>
<th>RhoA modification</th>
<th>Cellular function</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>TcdA/B</td>
<td>Clostridium difficile</td>
<td>Glucosylation</td>
<td>Disruption of epithelium barrier</td>
<td>Just et al. (1995); Chaves-Olarte et al. (1997)</td>
</tr>
<tr>
<td>TcsH</td>
<td>Clostridium sordelli</td>
<td>Glucosylation</td>
<td>Cell rounding up</td>
<td>Gent et al. (1996)</td>
</tr>
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<td>TcnA</td>
<td>Clostridium novyi</td>
<td>N-AcGlucosaminylation</td>
<td>Macrophage migration inhibition</td>
<td>Selzer et al. (1996); Rubin et al. (1988); Rotsch et al. (2012)</td>
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<tr>
<td>C3</td>
<td>Clostridium botulinum</td>
<td>ADP-ribosylation</td>
<td>Endothelium transcellular tunel</td>
<td>Boyer et al. (2006); Inoue et al. (1991)</td>
</tr>
<tr>
<td>EDIN-A/B/C</td>
<td>Staphylococcus aureus</td>
<td>ADP-ribosylation</td>
<td></td>
<td>Just, Schallehn and Aktories (1992)</td>
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<td>C3cer</td>
<td>Bacillus cereus</td>
<td>ADP-ribosylation</td>
<td></td>
<td>Von Pawel-Rammingen et al. (2000)</td>
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<tr>
<td>YopE</td>
<td>Yersinia spp.</td>
<td>GAP</td>
<td>Inhibition of phagocytosis</td>
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<tr>
<td>YopT</td>
<td>Yersinia spp.</td>
<td>Lipid anchor cleavage</td>
<td>Inhibition of phagocytosis</td>
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<td>VopS</td>
<td>Vibrio parahaemolyticus</td>
<td>AMPylation</td>
<td>Actin cytoskeleton collapse</td>
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<tr>
<td>ExoS, ExoT</td>
<td>Pseudomonas aeruginosa</td>
<td>GAP</td>
<td>Inhibition of phagocytosis, Actin cytoskeleton disruption</td>
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The pyrin inflammasome, probably by impairing the microtubule dynamics-mediated control. The molecular defects associated with the different autoinflammatory controlled by a microtubule-dependent mechanism targeted by colchicine. Hypermorphic mutations in the pyrin B30.2 domain decrease the threshold of activation of pyrin, thereby releasing its PYD domain and increasing the ability of pyrin to recruit ASC. Downstream of dephosphorylation, pyrin inflammasome activation is associated with release, and an inflammatory cell death termed pyroptosis. Pyrin may also directly sense perturbations in actin cytoskeleton dynamics as illustrated by PFIT syndrome associated with WDR1 mutations. In PAPA syndrome, mutations in PSTPIP1 increase the interactions between PSTPIP-1 protein and the inhibitory B-box domain of pyrin, thereby releasing its PYD domain and increasing the ability of pyrin to recruit ASC. Downstream of dephosphorylation, pyrin inflammasome activation is controlled by a microtubule-dependent mechanism targeted by colchicine. Hypermutotic mutations in the pyrin B3.2 domain decrease the threshold of activation of the pyrin inflammasome, probably by impairing the microtubule dynamics-mediated control. The molecular defects associated with the different autoinflammatory syndromes are numbered as follows: (1) FMF, (2) PAAND, (3) PAPA, (4) MKD, (5) PFIT.

The pyrin inflammasome is activated by various RhoA-inhibiting toxins, including TcdA and TcdB from C. difficile, VopS from Vibrio paraaeremolyticus, TecA from B. cenocepacia, the C3 toxin from C. botulinum, and YopE and YopT from Yersinia species (Xu et al. 2014; Aubert et al. 2016; Chung et al. 2016; Ratn et al. 2016). The diversity of toxins and effectors (Table 1) triggering pyrin inflammasome activation demonstrates that pyrin inflammasome does not result from sensing a specific RhoA modification. Accordingly, pyrin does not interact directly with RhoA. Pyrin senses a general impairment of RhoA activity that can result from either direct RhoA modifications, sequestration of RhoA in the cytosol or stimulation of its GTPase activity. The pyrin inflammasome displays specificity toward RhoA/B/C. Indeed, in 293T cells, RhoA, B and C are functionally redundant in controlling pyrin inflammasome activation downstream of TcdB (Xu et al. 2014). In contrast, the lethal toxin from C. sordelli, Tcsl, which glucosylates specifically RAS family and RAC proteins but not RhoA subfamily proteins, does not trigger pyrin inflammasome activation.

The molecular mechanisms controlling pyrin activation downstream of RhoA inactivation have been recently uncovered and involve the kinases PKN-1/2 (Park et al. 2016). PKN-1/2 are direct effectors of RhoA (Fig. 1). These two functionally redundant kinases dynamically phosphorylate pyrin on two serine residues (S208 and S242). As a consequence, phosphorylated pyrin interacts with chaperone proteins from the 14–3-3 family (also called YWHA-tyrosine 3-monoxygenase/tryptophan 5-monoxygenase activation proteins). 14–3-3 proteins sequester pyrin thereby blocking its ability to form an active inflammasome (Jen et al. 2005; Gao et al. 2016; Masters et al. 2016; Park et al. 2016). Inactivation of RhoA GTPases leads to a decrease in PKN-1/2 activity and finally results in the dephosphorylation of pyrin, its release from 14–3-3 proteins and the formation of an active pyrin inflammasome (Fig. 2).

While this mechanism appears to be central, pyrin inflammasome activation is also controlled by a microtubule-dependent mechanism downstream of this dephosphorylation step (Fig. 2). Indeed, drugs that destabilize microtubule dynamics (e.g. colchicine) inhibit pyrin inflammasome activation by TcdB while they do not affect pyrin dephosphorylation or its release from 14–3-3 proteins (Gao et al. 2016; Van Gorp et al. 2016). Microtubule dynamics appears important to promote the recruitment and the oligomerization of ASC, the core inflammasome adaptor protein (Gao et al. 2016; Van Gorp et al. 2016). Of note, the potential of drugs stabilizing the microtubule (paclitaxel) to inhibit the pyrin inflammasome remains controversial (Gao et al. 2016; Van Gorp et al. 2016) and deserves further investigations.

PKN-1/2 are direct RhoA effectors suggesting that pyrin senses RhoA inactivation upstream of the actin cytoskeleton dynamics regulatory mechanisms triggered by RhoA effectors. Yet, pyrin inflammasome activation is also observed in mice and patients bearing specific mutations in WDR1 (Kim et al. 2015; Kuhns
et al. 2016; Standing et al. 2017). WDR1 is a protein that promotes severing of filamentous actin and actin depolymerization. While, to our knowledge, the impact of these mutations on PKN-1/2 activity and pyrin phosphorylation has not been investigated, these findings indicate that pyrin inflammasome may be a general sensor of actin dynamic perturbations triggered by pathogens or sterile deregulations of host processes (Fig. 2).

INFLAMMASOMES AND YERSINIA: AN EXQUISITE EXAMPLE OF CO-EVOLUTION

The antibacterial role of an innate immune signaling pathway is best demonstrated when specific pathogens evolve effectors that specifically inhibit this pathway. This has been well exemplified, recently, by two very elegant studies on Y. pseudotuberculosis (Chung et al. 2016) and Y. pestis (Ratner et al. 2016). Yersinia spp. are extracellular pathogens that have evolved two T3SS effectors, YopE and YopT, to inhibit RhoA activity. YopE acts as a RhoA GAP (Black and Eliska 2000; Von Pawel-Rammingen et al. 2000), while YopT is a cysteine protease that cleaves the isoprenoid tail anchoring RhoA at the membrane (Zumbihl et al. 1999; Shao et al. 2002) (Fig. 1). These two effectors are important for the antiphagocytic activity of Yersinia spp. YopE and YopT prevent RhoA from interacting with PKN-1/2 at the plasma membrane, which should result in dephosphorylation of pyrin and activation of the pyrin inflammasome during Yersinia infection. Yet, activation of the pyrin inflammasome is undetectable during Y. pseudotuberculosis or Y. pestis infections and is only evident upon infection with ΔyopM mutant strains. Indeed, YopM inhibits the pyrin inflammasome. YopM acts as a scaffold protein in a multiprotein complex that includes pyrin and PKN-1/2. In vitro, YopM stimulates PKN-1/2-mediated pyrin phosphorylation indicating that during Yersinia infection, YopM directly hijacks PKN-1/2 to phosphorylate pyrin and inhibit inflammasome activation (Fig. 2). Thus, the virulence of Yersinia results from a sophisticated interaction of multiple effectors with the pyrin inflammasome clearly indicating a co-evolution of these pathogens with their mammalian hosts.

In parallel to pathogens evolution, certain human populations have selected mutations in the pyrin-encoding gene (MEFV) (Schaner et al. 2001), which confers to the pyrin inflammasome a decreased threshold of activation in response to RhoA-targeting toxins (Jamilloux et al. 2018) but can also be associated with an autoinflammatory syndrome termed familial Mediterranean fever (FMF) (French FMF Consortium 1997; The International FMF Consortium 1997).

MONOCENTIC AUTOINFLAMMATORY SYNDROMES

The inflammasomes are key for resisting microbial infections, yet mutations in inflammasome sensor-encoding genes are responsible for several hereditary autoinflammatory syndromes. The concept of autoinflammatory syndrome has been proposed in 1999 to define a group of diseases with abnormal innate immune system activation. Autoinflammatory syndromes are characterized by recurrent attacks of systemic inflammation with fevers and are distinguished from autoimmune diseases by the absence of autoantibody and autoantigen-specific T and B cells. Since 1999, about 30 monogenic autoinflammatory syndromes have been described, with mutations identified in genes implicated in innate immunity (McDermott et al. 1999; Manthiram et al. 2017). The best-described autoinflammatory syndromes are cryopyrin-associated periodic syndromes (CAPS), which involve gain-of-function mutations in the NLRP3 gene.

At least five monogenic autoinflammatory syndromes involve dysregulation of the pyrin inflammasome: familial Mediterranean fever (FMF), pyrin-associated autoinflammation with neutrophilic dermatosis (PAAND), pyogenic arthritis, pyoderma gangrenosum, acne (PAPA), mevalonate kinase deficiency (MKD), and autoinflammatory periodic fever, immunodeficiency, and thrombocytopenia (PFIT).

FAMILIAL MEDITERRANEAN FEVER

FMF is the most common monogenic autoinflammatory syndrome, affecting more than 100,000 individuals worldwide mostly from the eastern Mediterranean basin. It is characterized by recurrent, self-limited, episodes of fever and polyserositis. FMF is most often inherited in a recessive manner and the majority of FMF patients hold bi-allelic mutations in MEFV in a region encoding the B30.2 domain of pyrin. MEFV mutations are hypermorphic mutations with a gene-dosage effect (deleterious phenotypes increasing with the number of mutated alleles) (Omenetti et al. 2014; Jamilloux et al. 2018). We have recently demonstrated that FMF-associated MEFV mutations decrease the threshold of activation of the pyrin inflammasome in monocytes exposed to TcdB (Jamilloux et al. 2018). Interestingly, FMF-associated MEFV mutations also affect the NLRP3-dependent monocyte responses to LPS treatment suggesting intertwined connections between the pyrin and the NLRP3 inflammasomes (Omenetti et al. 2014). Yet, in contrast to the gain of function of NLRP3, NLR C4 or NLRP1 observed in CAPS, NLR C4- and NLRP1-associated inflammasomopathies (Hoffman et al. 2001; Canna et al. 2014; Kitamura et al. 2016; Zhong et al. 2016; Grandemange et al. 2017), FMF-associated MEFV mutations do not lead to a constitutive pyrin inflammasome activation and require a specific stimulus (e.g., low doses of TcdB) to trigger inflammasome activation (Omenetti et al. 2014; Van Gorp et al. 2016; Jamilloux et al. 2018).

Intriguingly, colchicine and various other inhibitors of microtubules dynamics, while highly efficient to block pyrin inflammasome activation in monocytes from healthy donors, are unable, in vitro, to inhibit TcdA-mediated pyrin inflammasome activation in monocytes from FMF patients. This result may indicate that FMF-associated mutations alleviate the poorly understood requirement for microtubule dynamics in the pyrin inflammasome activation cascade and suggest that the B30.2 domain is involved in this control (Van Gorp et al. 2016). The lack of microtubule dynamics-mediated control is likely responsible for the decreased threshold of activation of the pyrin inflammasome in FMF patients (Jamilloux et al. 2018). Since colchicine is the mainstay of FMF treatment, the in vivo inefficacy of colchicine to inhibit TcdA-mediated pyrin inflammasome in FMF patients was highly unexpected and this conundrum remains to be solved (Gao et al. 2016; Van Gorp et al. 2016).

PYRIN-ASSOCIATED AUTOINFLAMMATION WITH NEUTROPHILIC DERMATOSIS

In contrast to FMF, which is a disease mostly transmitted in a recessive mode, a different autoinflammatory syndrome, termed PAAND, is due to gain-of-function mutations in MEFV and is dominantly inherited. PAAND is characterized by combinations
of childhood-onset recurrent episodes of neutrophilic dermatosis, fever, elevated acute-phase reactants, arthralgia/arthritis and myalgia/myalgia (Masters et al. 2016).

Two distinct MEFV dominant mutations causing this rare syndrome have been identified (p.S242R and p.E244K). These mutations abolish or reduce PKN-1/2-mediated pyrin phosphorylation on serine 242 and impair the binding of 14–3–3 proteins leading to constitutive activation of the pyrin inflammasome. Importantly, this syndrome and the associated mutations demonstrate the importance and the relevance of this phosphorylation/dephosphorylation mechanism. PAAND is a more severe disease than FMF mirroring the intrinsic activity of the corresponding pyrin proteins. Indeed, PAAND-associated pyrin variants are constitutively active, while FMF-associated pyrin variants have a lower threshold of activation than the pyrin of healthy individuals.

Interestingly, PAAND-associated mutations do not map into the B30.2 domain, in which the majority of pathogenic FMF-associated mutations are found. Furthermore, FMF-associated MEFV mutations do not seem to affect in a major way pyrin phosphorylation level and 14–3–3 binding (Gao et al. 2016; Van Gorp et al. 2016; Moghaddas et al. 2017) in contrast to PAAND-associated MEFV mutations. These clinical and genetic observations on these two different diseases highlight the different regulatory domains of pyrin and reinforce the conclusion that (de)phosphorylation of pyrin is not the only process governing pyrin inflammasome activation.

While the two autoinflammatory syndromes described above are due to MEFV mutations, three other monogenic syndromes affect indirectly the pyrin inflammasome.

**PYOGENIC ARTHRITIS, PYODERMA GANGRENOSUM, ACNE**

PAPA syndrome is a dominantly inherited disease associated with missense mutations in the PSTPIP1 gene encoding the proline serine threonine phosphatase-interacting protein-1 (PSTPIP-1) (Wise et al. 2002). PAPA syndrome is characterized by recurrent arthritis and severe cutaneous symptoms including severe acne and nonhealing sterile ulcers.

PSTPIP-1 interacts with pyrin and promotes its ability to interact with ASC and to trigger its oligomerization. The current model indicates that, at steady state, pyrin is self-inhibited due to its inhibitory B-Box domain, which maintains the sensor in an inactive conformation by limiting the availability of its PYD domain to recruit ASC. PSTPIP-1 interacts with the B-box domain of pyrin, thereby releasing its PYD domain and increasing the ability of pyrin to recruit and oligomerize ASC (Yu et al. 2007) (Fig. 2). The ability of PSTPIP-1 to interact with pyrin is modulated by the level of PSTPIP-1 phosphorylation itself under the dual control of ABL-1 kinase and PEST-phosphatases. The roles of PSTPIP-1 and of this phosphorylation process in the response to RhoA-inhibiting toxins remain to be investigated. In PAPA syndrome, PSTPIP1 mutations decrease the ability of PSTPIP-1 to interact with PEST-phosphatase leading to hyper-phosphorylated PSTPIP-1 variants with a better avidity for pyrin (Shoham et al. 2003).

While there is clear evidence of a deregulation of inflammasome in PAPA syndrome (Omenetti et al. 2016), the pathophysiology of PAPA syndrome is complex and probably involves other functions of PSTPIP-1 besides its regulatory role on the pyrin inflammasome (Starnes et al. 2014). Furthermore, a large spectrum of autoinflammatory diseases associated with different PSTPIP1 mutations has been recently described and gathered under the term PAID for ‘PSTPIP1-associated inflammatory diseases’ (Holzinger and Roth 2016). The relevance of the deregulation of the pyrin inflammasome in the associated symptoms in relation with the different PSTPIP1 mutations remains to be demonstrated.

**MEVALONATE KINASE DEFICIENCY: AFFECTING RhoA ISOPRENYLATION**

MKD is a metabolic disease encompassing a continuum of two phenotypes, known as hyperimmunoglobulinemia D syndrome and mevalonic aciduria. Patients with MKD present with typical autoinflammatory symptoms including recurrent fever, arthralgia and skin rash. MKD is associated with bi-allelic mutations in the MVK gene leading to a profound reduction in mevalonate kinase activity (Drenth et al. 1999; Houten et al. 1999). This enzyme belongs to a metabolic pathway producing cholesterol and geranylgeranyl pyrophosphate. The latter is the precursor of geranylgeranyl lipid, which is covalently linked to the CAAX box of RhoA GTPases to target them to the membrane. MKD is associated with a reduction in geranylgeranyl pyrophosphate level and isoprenylation of Rho and Rab GTPases (Park et al. 2016). These mutations mimic the Yersinia YopT effector (which directly cleaves the RhoA isoprenylated tail), inhibit RhoA GTPases activity and lead to the activation of the pyrin inflammasome.

**AUTOINFLAMMATORY PERIODIC FEVER, IMMUNODEFICIENCY, AND THROMBOCYTOPENIA**

PFIT, described so far in a single family, is caused by homozygous missense mutations in WDR1. This disease has been associated with elevated IL-18 levels in the serum of patients (Standing et al. 2017). As previously described, WDR1 is a protein promoting F-actin severing and depolymerization. Interestingly, before the description of the first human cases, a similar autoinflammatory disease had been described in a mouse line homozygous for a hypomorphic wdr1 allele (Kim et al. 2015). The autoinflammatory symptoms were linked to a pyrin-dependent increase in IL-18 levels in the serum of these mice. Importantly, this disease illustrates for the first time that the pyrin inflammasome can be activated not only by pathogens’ toxins and effectors but also following specific perturbation of actin dynamics (Kim et al. 2015).

**CONCLUSION**

As exemplified here, numerous pathogens have evolved toxins and effectors to target RhoA GTPases while mammal hosts have evolved the pyrin inflammasome to detect inhibition of these critical host enzymes. Importantly, other innate immune signaling pathways such as the NOD1/RIP2/NF-κB pathway can be activated following sensing of Rho GTPases (e.g. Rac-1) deregulation (Fukazawa et al. 2008; Keesstra et al. 2013) or changes in actin dynamics (Bielig et al. 2014) indicating that while pathogens have evolved numerous effectors to target these cell biology hubs, the host itself has evolved several innate immune sensors and responses to detect this hijacking. Importantly, while the pyrin inflammasome is present only in mammals, effector-triggered immunity is a mechanism present throughout plants and animals evolution (Stuart and Boyer 2013).
There is emerging evidence that numerous pathogens have co-evolved with the pyrin inflammasome. The interaction of Yersinia species with mammalian hosts demonstrates such an exquisite co-evolution. The high carrier frequency of FMF in Mediterranean and Middle Eastern populations has long been suggested to be the result of a selective advantage conferred by FMF-associated MEFV alleles in resistance to an unknown pathogen (Schaner et al. 2001). It is thus tempting to speculate that FMF-associated pyrin variants were selected in specific human populations to confer protection against Yersinia or Yersinia-like pathogens (e.g. during plague epidemics) (Chung et al. 2016; Ratner et al. 2016). Nowadays, even though plague outbreaks are still observed (e.g. in 2017 in Madagascar; Roberts 2017), the possession of FMF-associated MEFV mutations seems mostly deleterious leading to detrimental exuberant autoinflammatory response and inflammatory syndromes. Interestingly, several autoinflammatory diseases are linked to deregulation of the pyrin inflammasome. The study of these pathologies and of their difference is likely to provide us novel insights on the fine mechanisms of pyrin activation in response to bacterial toxins and possibly in response to sterile danger signals.

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