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INSIGHTS

NLRP1 activation by UVB: Shedding light on an enigmatic inflammasome

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In this issue of *JEM*, Jenster et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20220837) investigate how UVB radiation promotes activation of the inflammatory immune sensor NLRP1, and in doing so uncover how NLRP1 recognizes a diverse range of ribotoxic stresses.

Inflammasomes are cytosolic multiprotein complexes that recognize and respond to a variety of pathogen- or environmental-associated triggers to induce a potent inflammatory response (Broz and Dixit, 2016). While many innate immune sensors directly recognize pathogen-associated ligands, inflammasomes are unusual in their ability to also detect disruptions in cellular homeostasis. While the mechanisms through which inflammasomes recognize pathogen-associated ligands are well defined, the mechanisms through which environmental triggers drive inflammasome activation have remained unclear. In this issue of *JEM*, Jenster et al. (2022) uncover the mechanism through which the human NLRP1 inflammasome is activated by UVB radiation. Through this work they also uncover that NLRP1 can sense ribotoxic stress induced by antibiotics and viral infection through a common signaling cascade.

The NLRP1 inflammasome is unusual, even among inflammasomes, due to the broad range of stimuli to which it responds and its atypical mechanism of activation. Similar to other NLR family members, NLRP1 encodes an N-terminal pyrin domain (PYD), a nucleotide-binding domain (NBD), and a leucine rich repeat (LRR). However, uniquely among NLRs, NLRP1 also encodes a C-terminal function to find domain (FIIND) followed by a caspase activation and recruitment domain (CARD). The NLRP1 FIIND undergoes autoproteolytic processing that separates NLRP1 into two non-covalently associated fragments. NLRP1 recognizes the activity of pathogen-encoded proteases through a mechanism deemed "functional degradation" (Sandstrom et al., 2019; Chui et al., 2019). Cleavage of NLRP1 by pathogen-encoded proteases promotes proteasomal degradation of the N-terminal domains and releases the processed C-terminal fragment. This liberated C-terminal fragment may then assemble into the active inflammasome, nucleating and promoting the oligomerization of the adaptor protein ASC into a large insoluble aggregate known as the ASC speck. Once assembled, the ASC speck recruits and activates the cytosolic protease casepase-1 (CASP1), which in turn processes the inflammatory cytokines IL-1β and IL-18 to their active isoforms and induces a lytic form of cell death known as pyroptosis.

While NLRP1 acts to sense pathogen-associated protease activity through this mechanism (Robinson et al., 2020; Tsu et al., 2021), cleavage is not the only know activator of NLRP1. NLRP1 can also sense disruptions in cellular homeostasis, including ATP depletion (Liao and Mogridge, 2013) and proteostatic stresses (Okondo et al., 2018; Zhong et al., 2018). Recent reports have also demonstrated that NLRP1 can sense viral infection through the recognition of double-stranded RNA (dsRNA; Bauernfried et al., 2021). How these different triggers promote the degradation of NLRP1 or whether they activate NLRP1 through a conserved mechanism has remained elusive. Finally, several reports have suggested that UVB radiation can induce activation of NLRP1 in human keratinocytes, but the mechanism by which NLRP1 could sense UVB remained unclear (Sand et al., 2018; Fenini, et al., 2018a).

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To reveal how NLRP1 could sense UVB radiation, Jenster et al. (2022) engineer a fluorescently labeled CASP1 CARD construct and measure incorporation into the ASC speck as a proxy for inflammasome activation in keratinocytes. The authors confirm NLRP1-dependent inflammasome activation after UVB exposure in these immortalized keratinocytes. Diving into the mechanism of activation, they demonstrate that neither

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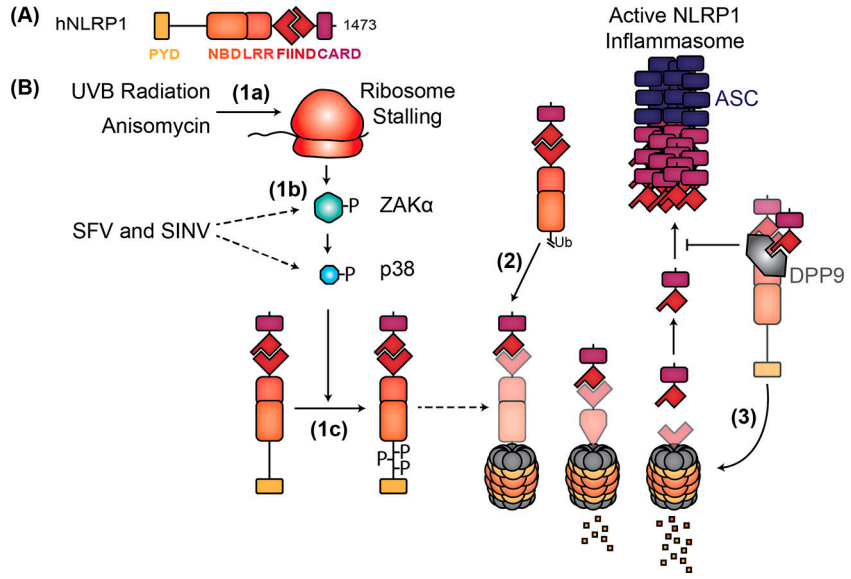

NLRP1 recognizes UVB radiation, ribotoxic stress, and viral infection via MAPK cascade. (A) Schematic of human NLRP1 domain architecture. (B) (1a) UVB radiation or certain antibiotics induce ribotoxic stress and activate ZAKα. (1b) Viral infection or CpG also induce activation of ZAKα. Phosphorylated ZAKα promotes phosphorylation of multiple p38 isoforms via MAPK signaling cascade. Active p38 phosphorylates the N-terminal linker between the PYD and NBD of NLRP1, promoting NLRP1 activation in a proteasome-dependent mechanism. Alternatively, neither activation of NLRP1 after cleavage and N-end rule mediated ubiquitination (Ub; 2) nor destabilization of the inhibitory DPP9 complex (3) require MAPK signaling to promote NLRP1 activation. SFV, Semliki Forst virus; SINV, Sinbdis virus.

DNA damage nor the generation of reactive oxygen species is sufficient to activate NLRP1 in this system, strongly suggesting that RNA damage induced by UVB is the trigger. UVB-induced RNA damage promotes the ribotoxic stress response mediated by the MAP kinase kinase kinase ZAKα (Wu et al., 2020; Vind et al., 2020). Following this lead, the authors find that activation of NLRP1 upon UVB exposure requires the activity of ZAKα and the downstream p38 kinases. Indeed, ribotoxic stress is sufficient to drive NLRP1 activation; keratinocytes treated with antibiotics that promote ribosome stalling and ZAKα phosphorylation rapidly induce NLRP1 dependent inflammasome activation. Consistent with the mechanism through which cleavage drives NLRP1 activation, activation of NLRP1 downstream of MAPK signaling also requires proteasomal activity.

Expanding on these results, the authors examine whether NLRP1 activation induced by viral infection requires MAPK signaling. In particular, infection with the alphaviruses Semliki Forst virus or Sinbdis virus is sufficient to induce NLRP1 activation. As observed with UVB, activation of NLRP1 by these viruses requires p38 activity and is partially dependent on ZAKα, suggesting that viral activation of NLRP1 is also mediated by MAPK signaling pathways. Further, NLRP1 activation by transfected dsRNA is also blocked by pharmacological inhibition of either ZAKα or p38. Critically, activation of NLRP1 by protease activity or inhibition of DPP9 does not require p38 activity, demonstrating distinct mechanisms of activation by these different triggers.

Examining the mechanism through which MAPK signaling promotes activation of NLRP1, the authors first demonstrate that ZAKα activity in and of itself is sufficient to drive NLRP1 activation via p38. Mutational analysis of NLRP1 further reveals that the unstructured linker between the N-terminal PYD and NBD is necessary for reactivity, and indeed that residues in this linker are directly phosphorylated by p38. This phosphorylation of NLRP1 is required for activation downstream of ribotoxic stress, as an NLRP1 construct in which all serines and threonines in the linker region are mutated to alanine is no longer sensitive to ribotoxic stress yet remains reactive to other stimuli. In total, these results demonstrate a novel mechanism through which NLRP1 surveys for cellular stresses and complement other recently published manuscripts that identified a role for MAPK signaling after UVB exposure in NLRP1 activation (Robinson et al., 2022; Fenini et al., 2018b).

These results clearly indicate that NLRP1 can act as a central signaling platform for immune surveillance of cellular homeostasis. Previous work had identified that NLRP1 could recognize pathogen-associated factors that directly induce the degradation of NLRP1 or proteostatic stresses that promoted the preferential degradation of NLRP1. This work uncovers a third arm of NLRP1 activation—phosphorylation by endogenous signaling pathways.

Still, many questions remain. Perhaps the primary mechanistic question is, how does phosphorylation of NLRP1 promote degradation and activation? Is phosphorylation sufficient to destabilize NLRP1, or are endogenous E3 ligases that recognize the phosphorylated NLRP1 linker recruited to promote ubiquitination and degradation of NLRP1? Additionally, how dsRNA and viral infection promote activation of ZAKα and p38 remains unclear. Finally, are there other endogenous kinases that can activate NLRP1 through parallel pathways? As such, several mechanistic details remain to be investigated further.

An interesting evolutionary question also arises from this work: When did NLRP1 begin to function as a sensor of MAPK activity? The authors find that the murine homologue of NLRP1 fails to recognize MAPK activity due to differences in the N-terminal linker region; however, it remains unclear whether p38 responsiveness was lost in the mouse or gained in human. Further, whether NLRP1 functions primarily as a sensor of pathogen-associated activities or as a general sensor of cellular stress remains a point of contention in the field. Here the authors find that recognition of both pathogen and environmental stresses are mediated through a shared pathway. But is inflammation in response to UVB radiation due to an inappropriate antiviral response? Or has sensing of an environmental stress been co-opted to protect against viral infection? These larger questions of how NLRP1 promotes health and immunity against both environmental and pathogenic stresses in the skin and other tissues remain important areas of future study.

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References

Bauernfried, S., et al. 2021. *Science*. https://doi.org/10.1126/science.abd0811

Broz, P., and V.M. Dixit. 2016. *Nat. Rev. Immunol.* https://doi.org/10.1038/nri.2016.58

Chui, A.J., et al. 2019. *Science*. https://doi.org/10.1126/science.aau1208

Fenini, G., et al. 2018a. *J. Invest. Dermatol.* https://doi.org/10.1016/j.jid.2018.07.016

Fenini, G., et al. 2018b. *J. Invest. Dermatol.* https://doi.org/10.1016/j.jid.2017.10.037

Jenster, L.-M., et al. 2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20220837

Liao, K.C., and J. Mogridge. 2013. *Infect. Immun.* https://doi.org/10.1128/IAI.01003-12

Okondo, M.C., et al. 2018. *Cell Chem. Biol.* https://doi.org/10.1016/j.chembiol.2017.12.013

Robinson, K.S., et al. 2020. *Science*. https://doi.org/10.1126/science.aay2002

Robinson, K.S., et al. 2022. *Science*. https://doi.org/10.1126/science.abl6324

Sand, J., et al. 2018. *Cell Death Dis.* https://doi.org/10.1038/s41419-017-0009-4

Sandstrom, A., et al. 2019. *Science*. https://doi.org/10.1126/science.aau1330

Tsu, B.V., et al. 2021. *Elife*. https://doi.org/10.7554/eLife.60609

Vind, A.C., et al. 2020. *Mol. Cell*. https://doi.org/10.1016/j.molcel.2020.03.021

Wu, C.C., et al. 2020. *Cell*. https://doi.org/10.1016/j.cell.2020.06.006

Zhong, F.L., et al. 2018. *J. Biol. Chem.* https://doi.org/10.1074/jbc.RA118.004350