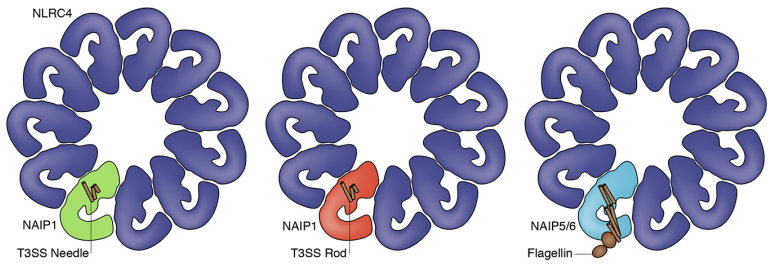
Down with doublespeak: NAIP/NLRC4 inflammasomes get specific

Insight from Youssef Aachoui (left) and Edward Miao (right)

The NAIP family members are cytosolic pattern recognition receptors that detect the activity of bacterial type III secretion systems (T3SSs) when they aberrantly translocate one of three NAIP ligands. Mouse NAIP1 and NAIP2 bind the T3SS needle and rod proteins, whereas NAIP5 and NAIP6 bind flagellin. T4SSs are also detected when they cause flagellin to enter the cytosol. Upon interaction with its ligand, a single NAIP molecule changes its conformation to interact with and activate NLRC4, which propagates the signal to the next NLRC4, leading to the formation of disk-shaped oligomeric complexes. This inflammasome hub contains a single NAIP polymerized with 9–11 NLRC4 proteins. Thereafter, it activates caspase-1 to cleave the cytokines IL-1β and IL-18 into their mature forms and also cleaves gasdermin D (GSDMD) to trigger pyroptosis (a form of inflammatory programmed cell death). Despite in vitro biochemical studies that described the molecular mechanism of activation of the NAIP/NLRC4 inflammasome, in vivo genetic evidence of the NAIP specificity remained to be published.

In this issue, Rauch et al. and Zhao et al. provide convincing genetic evidence showing the specificity of NAIPs to detect their predicted ligands. Previously, the examination of NAIPs in vivo was largely obstructed by the challenge to generate single NAIP-deficient mice because of the homology between *Naip* genes. Recent advances in CRISPR/Cas9 and TALEN targeting strategies lifted this hurdle, even allowing simultaneous targeting of *Naip* genes with a single guide RNA. In these two studies, the authors generated *Naip1*$^{-/-}$, *Naip2*$^{-/-}$, *Naip5*$^{-/-}$, and *Naip1-6*$^{\Delta/\Delta}$ mice. Using retroviruses (in vitro) or toxin lethal factor (LFn) proteins (in vitro and in vivo) as delivery systems for NAIP ligands, the investigators demonstrated that loss of NAIP1 or NAIP2, as predicted, resulted in failure to detect T3SS needle or rod proteins, respectively. More importantly, similar to the lethality of LFn-flagellin caused by excessive NLRC4 activation in vivo, the authors showed that LFn-needle and LFn-rod toxins are lethal and that *Naip1*$^{-/-}$ and *Naip2*$^{-/-}$ mice are protected from their corresponding ligand. Biochemical studies indicated that flagellin is detected by NAIP5 and NAIP6. Consistent with this, *Naip5*$^{-/-}$ mice have significantly disrupted LFn-flagellin responses, but only *Naip1-6*$^{\Delta/\Delta}$ mice are fully protected, pointing to an in vivo role of *Naip6*. Finally, using engineered *Salmonella* Typhimurium to express a single ligand, Zhao et al. demonstrated the specificity of NAIP detection during infection in vivo.

The current works shed light on the NAIP/NLRC4 redundancy during detection and its implication in bacterial clearance of cytosolic pathogens such as *Salmonella*, *Burkholderia*, *Chromobacterium*, *Legionella*, *Pseudomonas*, and *Shigella*. Although each NAIP detects a specific ligand, they all converge to activate NLRC4. Thus, each ligand stimulates the same response, consistent with cytosolic flagellin, rod, and needle proteins being markers of the same type of cytosolic contamination event by T3SSs or T4SSs.



The NAIP/NLRC4 inflammasomes detect specific ligands. NAIP1 and NAIP2 detect the T3SS needle and rod proteins, respectively, whereas, NAIP5 and NAIP6 detect flagellin. Upon interaction with its ligand, a single NAIP recruits and activates one NLRC4, which propagates the signal to further NLRC4 molecules leading to the formation of disk-shaped oligomeric complexes composed of 1 NAIP and 9–11 NLRC4 proteins.

The redundancy within the NAIP subfamily to detect three different ligands as markers for the same T3SS/T4SS cytosolic contamination event raises questions about the evolution of the NAIP/NLRC4 inflammasome. In inbred mice, C57BL/6 mice express four functional NAIPs (NAIP1, 2, 5, and 6), whereas 129 mice additionally encode *Naip4* and *Naip7*, whose function is yet to be determined. In contrast, humans express a single NAIP that can detect T3SS needle protein or flagellin via two different splice forms, but apparently, humans cannot detect T3SS rod protein. Furthermore, the human NAIP locus is littered with remnants of NAIP genes. Why would these apparently similar functions be conferred by differently organized genes in an apparent example of paradoxical divergent then convergent evolution? Why are there no NAIP sensors for T4SS apparatus proteins? Do additional NAIPs detect different ligands in other mice or in other animal species? If so, they would require exquisite signal to noise ratios, as the NAIP/NLRC4 inflammasome can theoretically detect a single molecule of each specific protein ligand in the cytosol. Perhaps the dynamic nature of the NAIP locus is driven by constant exposure to certain pathogens that express selective NAIP ligands. Regardless of these outstanding questions, Rauch et al. and Zhao et al. provide in vivo proof of ligand specificity that makes NAIP/NLRC4 one of the best understood of all inflammasomes.

Rauch, I., et al. 2016. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20151809
Zhao,Y., et al. 2016. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20160006

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