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How bacteria can block xenophagy: an insight from *Salmonella*

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ABSTRACT

Xenophagy, a unique type of selective macroautophagy/autophagy, targets invading pathogens as part of the host immune response. In order to survive within the host, bacteria have established various self-defense mechanisms. In a recent paper from Feng Shao's lab, the *Salmonella* effector protein SopF has been demonstrated to block xenophagy by interrupting the vacuolar type H^+-translocating (v-) ATPase-ATG16L1 axis, which is important for antibacterial autophagy initiation. SopF can specifically ADP-ribosylate Gln124 on ATP6V0C, a v-ATPase component, thus influencing recruitment of ATG16L1 onto the bacteria-containing vacuole within the host cytosol.

Abbreviations: ATG: autophagy-related; S. Typhimurium: *Salmonella enterica* serovar Typhimurium; T3SS: type III secretion system

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There is a continual battle between host and microbe, in which both are competing for supremacy [1]. Xenophagy plays an important role in the immune system response against bacterial invasion, while bacteria are evolving to escape from, block or even subvert this antibacterial degradation pathway. For example, previous studies have found that *Shigella flexneri* has 2 type III secretion system (T3SS) effector proteins, VirA and IcsB, which can indirectly interrupt auto-phagy recognition through different mechanisms [2,3]. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is a model pathogen that is widely used for investigating how the host recognizes intracellular bacteria, but one problem with using *S.* Typhimurium to study xenophagy is that the autophagic response to its invasion is not robust [4]. To investigate the possible mechanism behind this inefficient autophagy when *Salmonella* invades a host cell, Xu et al. started with an unbiased screen to identify a bacterial effector protein, and revealed a connection between this protein with xenophagy components [5].

Using the colocalization of GFP-LC3 with bacteria as the readout, the authors found a mutant from the screen that shows a clear increase in xenophagy activity. The corresponding gene, *sopF*, had been recently identified [6]. In the study by Xu et al., the deletion of bacterial *sopF* increases antibacterial autophagy activity, and the overexpression of SopF in Δ*sopF* cells abolishes this phenotype. SopF is secreted by the T3SS [6], and there are 2 T3SSs in *S.* Typhimurium, encoded by the *Salmonella* pathogenicity islands (SPI) 1 and 2 [7]. The authors undertook a follow-up TEM1 translocation assay to demonstrate that SopF is an SPI-1 effector. They also found that the protein mainly localizes in the cytosol once *Salmonella* enters the host cell. Furthermore, the inhibition by SopF is broad-spectrum, facilitating infection by multiple bacterial species, whereas the inhibitory effect is specific for xenophagy and does not block nonselective autophagy.

Next, the authors analyzed the mechanism of SopF action. They found that RB1CC1/FIP200 is not required for the autophagic response when *sopF* is knocked out. Accordingly, they carried out a fluorescence-activated cell sorting-based genome-wide CRISPR-Cas9 screen in RB1CC1$^{-/-}$ HeLa cells infected with Δ*icsB* Δ*virA S. flexneri*, looking for cells that demonstrated decreased GFP-LC3 puncta formation. Results from the screen revealed that most essential autophagy-related (*ATG*) genes are required for xenophagy of *S. flexneri*. Importantly, a cluster of 5 genes encoding v-ATPase subunits was also identified, and subsequent experiments verified the role of the v-ATPase in xenophagy initiation. Mass spectrometry following affinity isolation of ATP6V1A, a major v-ATPase subunit, identified 2 components of the autophagy ubiquitin-like conjugation systems, ATG5 and ATG16L1, as ATP6V1A-interacting partners specifically under conditions of bacterial infection.

By monitoring fluorescently tagged ATG16L1, the authors found that the v-ATPase is involved in recruiting this protein to the bacteria-containing vacuole, where it plays a role in LC3 lipidation. The infection-induced recruitment of ATG16L1 to the v-ATPase is sensitive to the presence of SopF. ATG16L1 contains a WD40 domain that is not required for nonselective autophagy [8]. ATG16L1 mutants lacking this part of the protein, however, are defective in binding the v-ATPase and in xenophagy of Δ*icsB* Δ*virA S. flexneri*, in agreement with the observation that the host response to Δ*sopF* bacteria does not require RB1CC1 (i.e., canonical autophagy). Additional mutagenesis studies indicate that ATG16L1 homo-dimerization, which is mediated by the coiled-coil domain, is important for xenophagy.

The results from this study suggest that SopF can block the v-ATPase-ATG16L1 interaction without affecting the v-ATPase's proton pumping activity. In addition, data

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from a mouse model further support the hypothesis that SopF can promote the growth of *Salmonella* by blocking autophagy. Finally, the authors obtained a crystal structure for SopF, which indicates that it is a member of the ADP-ribosyltransferase family. Based on this finding, they further identified a component of the v-ATPase, ATP6V0C, as a substrate of SopF. Mass spectrometry revealed Gln124 as the target of ADP-ribosylation, and mutagenesis of this site prevents v-ATPase-dependent ATG16L1 recruitment to bacteria-containing vacuoles and the xenophagic response, but again does not interfere with nonselective autophagy.

The paper by Xu et al. not only reveals a previously unknown connection between the *Salmonella* effector protein SopF with xenophagy, but also uncovers an unexpected role of the v-ATPase in sensing intracellular bacteria for xenophagy induction.

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