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LRSAM1, an E3 Ubiquitin Ligase with a Sense for Bacteria

Jean Celli[1,*]

[1]Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT 59840, USA

Abstract

Antimicrobial autophagy is a host cellular process that captures and delivers intracellular parasites to lysosomes following their targeting as cargo via ubiquitination. Huett et al. (2012) show that the LRR- and RING-domain-containing E3 ubiquitin ligase LRSAM1 recognizes various bacteria and generates a ubiquitin signal that initiates the autophagic cascade.

Autophagy is a conserved eukaryotic cellular process that plays major roles in cellular homeostasis and innate immune control of intracellular microbes (Levine et al., 2011). Mechanistically, autophagy consists of the physical capture of cytosolic cargo into double-membrane vesicles for delivery to the lysosomal compartment for degradation. Selective autophagy targets the capture of specific organelles, protein aggregates, or invading microorganisms and requires specific recognition of the cargo to be targeted. Antibacterial autophagy can be directed toward either cytosolic or vacuolar bacteria and has been demonstrated for various intracellular pathogens including *Mycobacterium tuberculosis, Listeria monocytogenes, Shigella flexneri*, and *Salmonella* Typhimurium (reviewed in Knodler and Celli, 2011). Although typically considered a vacuolar parasite, *Salmonella* has become a useful model pathogen to study antibacterial autophagy, since a fraction of intracellular *Salmonella* disrupts its vacuole and reaches the cytosol in epithelial cells, allowing bacterial detection by the host cell and targeting for autophagic degradation (Birmingham et al., 2006). A dominant pathway in the autophagic capture of *Salmonella* relies on tagging bacteria with a poly-ubiquitin coat, which is then bound by ubiquitin-binding autophagy adaptors, such as SQSTM1/p62 or NDP52 (Birmingham et al., 2006; Cemma et al., 2011; Thurston et al., 2009; Zheng et al., 2009). These adaptors subsequently recruit specific autophagic machinery components, such as the GABARAP/LC3/ATG8 family proteins, triggering the autophagic cascade and autophagosome formation. Despite our increasing knowledge of the molecular aspects of the antibacterial autophagic cascade, a major unanswered question in the field is how the host cell senses bacteria in the cytosol and tags them with ubiquitin to initiate the autophagic cascade. Huett et al. (2012) now provide a response by identifying a mammalian E3 ubiquitin ligase, LRSAM1, that possesses a leucine-rich repeat (LRR) domain capable of recognizing bacteria (Figure 1).

In a search for novel innate immune sensors, LRSAM1 was recently identified by the same laboratory via protein interaction network analysis of human LRR domain-containing proteins and shown to be required for antibacterial autophagy (Ng et al., 2011). Further characterization of this protein has now shown its association in epithelial cells with both Gram-negative or Gram-positive bacteria targeted for autophagy. Importantly, siRNA-mediated depletion of LRSAM1 increased the numbers of cytosolic *Salmonella* to the same extent as depletion of the autophagy-related protein ATG16L1, underscoring its role in the

*Correspondence: jcelli@niaid.nih.gov.

autophagic control of bacteria. While a deletion of its LRR domain prevented LRSAM1association with bacteria, a RING domain-deleted LRSAM1 was still recruited to the vicinity of cytosolic *Salmonella* but did not generate ubiquitination (Huett et al., 2012). This clearly assigned a bacterial recognition role to the LRR domain and the ubiquitin ligase function to the RING domain. LRSAM1 bound to the autophagy adaptor NDP52 via its LRR domain, further linking this protein to the autophagic cascade, but not to SQSTM1/p62, GABARAP, or LC3. Yet, LRSAM1 association with bacteria was independent of NDP52 but was required for stable recruitment of the ubiquitin-binding adaptors NDP52 and SQSTM1/p62, suggesting that LRSAM1 provides the ubiquitin signal required for recruitment of these adaptor proteins. LRSAM1 function is specific to the ubiquitin-dependent pathway, as diacylglycerol (DAG)-mediated autophagy was not affected by depletion of LRSAM1. Additionally, the authors validated their siRNA-mediated depletion-based results with the demonstration that lymphoblasts from naturally LRSAM1-deficient individuals failed to control intracellular proliferation of *Salmonella* while retaining their ability to target protein aggregates to autophagy, further exemplifying the favored role of LRSAM1 in antibacterial autophagy (Huett et al., 2012).

A key finding of this study is the in vitro reconstitution of bacterial ubiquitination by purified LRSAM1 in the presence of specific E1 and E2 enzymes and ubiquitin. The demonstration of ubiquitin association with bacteria in absence of other host factors argues that LRSAM1 is an E3 ubiquitin ligase capable of bacterial recognition. Whether the ubiquitin signal associated with bacteria is provided by autoubiquitinated LRSAM1 or by LRSAM1-mediated ubiquitination of bacterial surface components remains to be determined. The observed promiscuity of LRSAM1-driven ubiquitination of phylogenetically unrelated bacteria in an in vitro context argues for either a specific ubiquitination of conserved bacterial surfaces components or motifs or a nonspecific ubiquitination of accessible bacterial molecules. Yet, it is not possible to rule out that autoubiquitination of bacteria-associated LRSAM1 provides the ubiquitin signal that triggers the autophagic cascade, a scenario that would more easily account for LRSAM1 promiscuous activity and would underscore the major role of LRR domain-mediated bacterial recognition in providing targeting signals for autophagic capture.

LRSAM1-mediated direct ubiquitination of bacteria was quite inefficient when reconstituted in vitro, so it remains possible that additional yet-to-be-identified host factors contribute to bacterial ubiquitination mediated by LRSAM1 in whole cells. LRSAM1-driven polyubiquitination in vitro favored unusual K6 and K27 linkages, which may define some specificity toward antibacterial autophagy. Yet, additional polyubiquitin linkages have been observed around *Salmonella* in infected cells (van Wijk et al., 2012) and residual ubiquitin association with *Salmonella* persists in LRSAM1-depleted cells (Huett et al., 2012), suggesting that additional LRSAM1-independent ubiquitination events occur in cells, possibly through the action of (an)other E3 ubiquitin ligase(s). Interestingly, LRSAM1 activity on bacteria was qualitatively promiscuous under in vitro reconstitution conditions, since it generated ubiquitination of strains of *L. monocytogenes* and *S. flexneri* that normally do not associate with LRSAM1 and avoid ubiquitination and autophagic recognition intracellularly. Autophagy avoidance mechanisms of these bacteria may require intracellular activation and specific host factors absent in these assays. Yet, the lack of LRSAM1 association with intracellular *Shigella* is intriguing and suggests that this bacterium possesses mechanisms to prevent its recognition by LRSAM1, a stage in the autophagic cascade that normally precedes ATG5 binding that is inhibited via secretion of IcsB (Ogawa et al., 2005). Indeed, as we discover novel facets of innate immune mechanisms that control intracellular parasites, it is likely that microorganisms have evolved counteracting strategies that await discovery. It will be most interesting to determine

whether cytosolic pathogens display intrinsic ways to prevent their recognition by LRSAM1 or perhaps other similarly acting sensors.

While the discovery of LRSAM1 function in antibacterial autophagy is a major leap in our understanding of innate immune sensing of bacteria in the host cytosol, it also brings additional questions: What pattern(s) does the LRSAM1 LRR domain recognize on cytosolic bacteria? Are other innate immune sensors and/or E3 ubiquitin ligases involved in recognition and tagging of bacterial targets for autophagy? How are membrane-bound bacteria subjected to autophagic control sensed? Do other innate sensors recognize these parasitic structures? Do they recognize the pathogen's molecules exposed on the vacuolar membrane or host molecules acting as signatures of pathogenic vacuoles (danger signals)? Future studies will undoubtedly uncover additional levels of complexicity in both the cytosolic mechanisms of pathogen sensing and the counteracting strategies developed by cytosol-adapted microbes.

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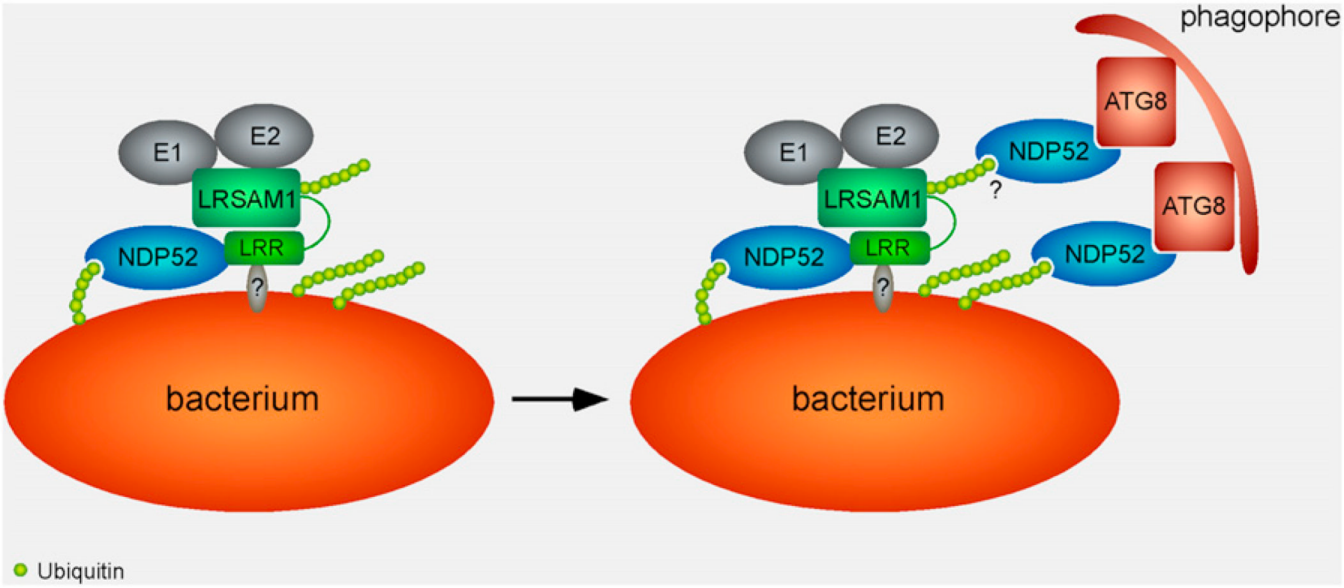

Figure 1. LRSAM1 Direct Recognition of Cytosolic Bacteria Triggers Autophagic Capture

LRSAM1 recognizes unknown component(s) on the surface of a cytosolic bacterium via its LRR domain, autoubiquitinates, and triggers ligation of polyubiquitin chains on bacterial components in combination with dedicated E1 and E2 enzymes. LRSAM1 also binds the autophagy adaptor NDP52, which in turn recognizes polyubiquitin chains and binds ATG8 family autophagy proteins, therefore triggering the autophagic cascade and bacterial capture into an autophagosome.