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Legionella pneumophila evades host-autophagic clearance using phosphoribosyl-polyubiquitin chains

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Legionella pneumophila, the causative agent of Legionnaires' disease, colonises host cells through effective avoidance of host defense mechanisms. In this commentary, we highlight two recent Nature Communications studies reporting that two Legionella effectors, Sdc and Sde, working together to decorate the Legionella-containing vacuole (LCV) with unusual mixed ubiquitin chains. The phosphoribosyl modifications on the polyubiquitin chains block recognition by ubiquitin adaptors, thereby preventing the recruitment of host autophagy receptors like p62 to the LCV. This allows Legionella to evade autophagic clearance and establish a replicative niche within host cells.

Legionella pneumophila catalyzes unusual phosphoribosyl-serine ubiquitination

The SidE family of effector proteins (SidEs) from *Legionella pneumophila* catalyze a novel form of ubiquitination independent of the canonical E1-E2-E3 enzyme cascade[1,2]. First, the mono-ADP-ribosyltransferase (mART) domain of SidEs catalyzes the transfer of ADP-ribose from NAD^+ to arginine 42 of ubiquitin, generating ADP-ribosylated ubiquitin (ADPR-Ub). The phosphodiesterase (PDE) domain then processes ADPR-Ub to phosphoribosylated ubiquitin (PR-Ub) by cleaving the pyrophosphate bond and releasing AMP. The PDE domain then catalyzes the conjugation of PR-Ub to serine residues in substrate proteins via a phosphodiester bond. This occurs through a two-step mechanism: first, PR-Ub is linked to a catalytic histidine in the PDE domain via a phosphoamidate linkage, forming a covalent intermediate. Secondly, the phosphoribosyl-ubiquitin is transferred from the catalytic histidine to a serine hydroxyl in the substrate protein[3–5].

The activity of the SidE effectors is tightly regulated by other Legionella effectors. SidJ, a metaeffector, inhibits SidE-mediated PR-ubiquitination by glutamylating the mART domain catalytic residue E860, thereby blocking the initial step of ADPR-Ub generation. SidJ glutamylation activity requires the eukaryotic co-factor calmodulin and is activated by decreased cytosolic Ca^{2+} levels[6–8]. Additionally, DupA and DupB, two Legionella effectors with PDE domains homologous to SidEs, act as specific deubiquitinases that reverse PR-serine ubiquitination[9,10]. Despite the structural similarity, DupA/B exhibit higher affinity for PR-ubiquitinated substrates compared to SidEs. This enables DupA/B to specifically cleave the phosphodiester bond between PR-Ub and serine residues, counteracting SidE activity. The balance between SidE-mediated PR-ubiquitination and DupA/B-mediated deubiquitination dynamically regulates the extent and duration of this unique post-translational modification during Legionella infection.

Legionella pneumophila SidC/SdcA are ubiquitin ligases that facilitate bacterial phagosomal remodeling

Another major family of *L. pneumophila* ubiquitin ligases is the Sdc proteins (SidC and SdcA). In contrast to the SidE family, the Sdc effectors catalyze conventional lysine-targeted ubiquitination via a novel E3 ubiquitin ligase domain. Initially, SidC and SdcA were proposed to act as vesicle tethering factors that recruit ER-derived vesicles to the LCV[11]. However, structural and biochemical studies have revealed that SidC and SdcA possess E3 ubiquitin ligase activity, defining a novel family of bacterial ubiquitin ligases[12,13]. SidC and SdcA each contain an N-terminal SNL (SidC N-terminal Ligase) domain and a C-terminal PI4P-binding domain that anchors them to the LCV membrane. The crystal structure of the SNL domain revealed a unique fold with no homology to known ubiquitin ligases. Intriguingly, the SNL domain contains a canonical catalytic triad consisting of a cysteine, histidine, and aspartate residue, reminiscent of the active site found in cysteine proteases and deubiquitinases. During infection, SidC/SdcA-mediated ubiquitination is important for recruitment of ER proteins and ubiquitin to the LCV. A SidC mutant lacking E3 ligase activity fails to complement these recruitment defects in a ΔsidC-sdcA strain. SidC and SdcA appear to ubiquitinate different substrates, as they exhibit distinct preferences for ubiquitin-conjugating E2 enzymes. This functional diversification likely allows Legionella to maximize its manipulation of host ubiquitination pathways.

The discovery of the SidC/SdcA E3 ligases expands the repertoire of strategies used by Legionella to hijack host cells. By combining ubiquitin ligase activity with phospholipid binding and vesicle tethering capabilities, these novel effectors dynamically reshape the phagosomal membrane to promote Legionella survival and replication.

SidEs and Sdc cooperate to avoid recognition by host-autophagic adaptors

The recent studies by Kotewicz et al. and Wan et al. have provided novel insights into the intricate interplay between the SidE and Sdc effectors in shaping the unique ubiquitin landscape on the LCV[14,15] (Fig. 1). Through a combination of biochemical, cell biological, and structural approaches, these studies have unveiled a remarkable cooperative action between these two effector families, leading to the decoration of the LCV with unusual mixed ubiquitin chains.

Kotewicz et al. focused on dissecting the role of the SidE effectors' deubiquitinase (DUB) domain, which had previously been shown to preferentially cleave K63-linked polyubiquitin chains. Surprisingly, they found that the DUB domain, rather than reducing polyubiquitination on the LCV, actually promotes the PR-ubiquitination of Rtn4 and the subsequent ER remodeling. Through a series of elegant experiments, they demonstrated that the DUB domain's activity is critical for generating monoubiquitin from K63-linked chains, which in turn serves as a substrate for the mART domain to generate ADPR-Ub. This ADPR-Ub is then used by the PDE domain for PR-ubiquitination of Rtn4. These findings provide a novel mechanistic understanding of

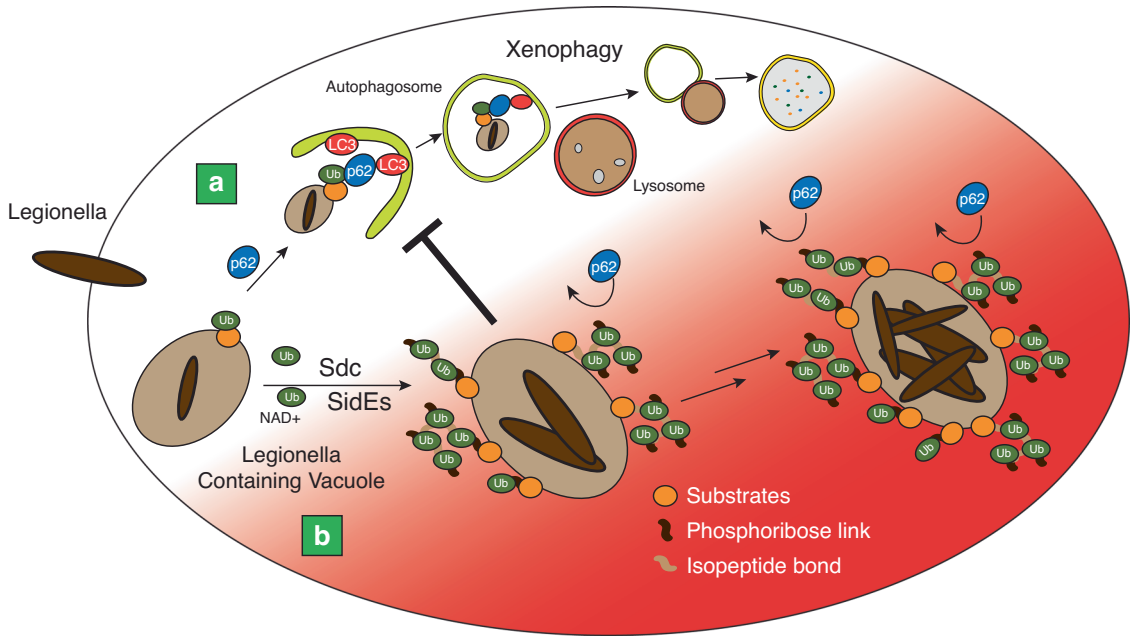

Fig. 1 | Phosphoribose-polyubiquitin chain building on LCV by Sdc and SidEs prevents autophagic clearance of Legionella pneumophila. a In general, intracellular bacteria such as Legionella are recognized by host-autophagic adaptor molecules, such as p62. The interaction between p62 and ubiquitin on the bacteria is required for guiding the pathogen to the autophagosome. Following fusion of the autophagosome and lysosome, the bacteria are cleared from the cytosol. **b** Legionella pneumophila avoids host-autophagic clearance through the cooperative action of the Sdc and SidE effector families. Upon internalization, Legionella resides within the Legionella-containing vacuole (LCV). The bacterial effectors Sdc and SidEs are translocated into the host cell, where they work together to modify the ubiquitin landscape on the LCV surface. Sdc catalyzes conventional polyubiquitination on host substrates, while SidEs perform phosphoribosyl-linked serine ubiquitination (PR-ubiquitination). Furthermore, SidEs modify the polyubiquitin chains assembled by Sdc by adding PR moieties to the Arg42 residue of ubiquitin. This coordinated action of Sdc and SidEs generates a unique ubiquitin code on the LCV, consisting of mixed ubiquitin chains with both conventional and PR linkages, as well as PR-modified ubiquitins within the polyubiquitin chains. The modification of the polyubiquitin chains by SidEs prevents recognition by ubiquitin-binding autophagy adaptors like p62, thereby allowing L. pneumophila to evade autophagic clearance via xenophagy. As a result, the LCV matures into an ER-derived replicative niche, promoting bacterial survival and growth.

how the different domains within SidE effectors cooperate to fine-tune the ubiquitin signals on the LCV.

Wan et al., on the other hand, took a broader approach to investigate the crosstalk between the SidE and SdC effectors. Through a combination of immunoprecipitation and mass spectrometry analyses, they discovered that multiple host proteins, including Rab small GTPases and ER proteins, are co-modified by both conventional ubiquitination (mediated by SidC) and PR-ubiquitination (mediated by SidE). This co-modification was found to be crucial for the efficient recruitment of these host proteins to the LCV and the subsequent remodeling of the ER. Notably, they demonstrated that SidC and SidE effectors work cooperatively to build mixed ubiquitin chains, where SidC extends polyubiquitin chains on substrates initially modified with PR-Ub by SidE. This intricate collaboration between the two effector families highlights the sophisticated strategies employed by L. pneumophila to fine-tune the host-pathogen interface.

A key finding that emerged from both studies is the identification of a novel mechanism by which L. pneumophila evades autophagic recognition and clearance. Through mass spectrometry analysis, Kotewicz et al. and Wan et al. discovered that SidE effectors specifically modify the R42 residue of ubiquitin within polyubiquitin chains on the LCV. This modification is of particular significance, as the R42 residue is a critical determinant for ubiquitin recognition by autophagy adaptors such as p62. By modifying this residue, SidE effectors sterically hinder the binding of p62 to ubiquitin, effectively preventing the recruitment of autophagy receptors to the LCV. This discovery provides a mechanistic explanation for how L. pneumophila can maintain an extensively ubiquitinated vacuole while avoiding autophagic degradation.

Another intriguing finding from Wan et al. is the ability of SidE effectors to generate PR-Ub linkages within polyubiquitin chains, leading to the crosslinking of multiple substrates into ubiquitin-tethered complexes on the LCV. They demonstrated this phenomenon by identifying complexes containing Rab1, Rab33b, and the ER protein LULL1. This additional layer of complexity in the ubiquitin architecture on the LCV further underscores the intricate strategies employed by L. pneumophila to create a specialized replicative niche.

Furthermore, both studies shed light on the role of additional players in the PR-ubiquitination process. Wan et al. identified the involvement of the PR-Ub deubiquitinases DupA and DupB in converting ADPR-Ub to PR-Ub within polyubiquitin chains. This finding expands our understanding of the enzymatic repertoire that L. pneumophila employs to fine-tune the ubiquitin signals on the LCV and highlights the dynamic nature of these modifications.

Perspectives

The findings by Kotewicz et al. and Wan et al. have significantly advanced our understanding of the sophisticated strategies employed by L. pneumophila to manipulate the host ubiquitin system. These

studies have unveiled a remarkable level of cooperation and crosstalk between the SidE and SidC effector families, leading to the generation of distinctive mixed ubiquitin chains on the LCV. The discovery that these effectors collaborate to decorate the LCV with PR-ubiquitin marks, thereby camouflaging the vacuole from autophagic recognition, represents a novel mechanism by which a pathogen can subvert host defenses and ensure its survival and replication.

The identification of this intricate ubiquitin manipulation strategy raises intriguing questions and opens up new avenues for future research. A key area of interest is to understand the temporal and spatial regulation of SidE and SidC activities during the course of infection. Deciphering the mechanisms that govern the coordinated action of these effectors will provide valuable insights into how L. pneumophila fine-tunes the host-pathogen interface at different stages of its intracellular lifecycle. Additionally, structural studies aimed at elucidating the molecular basis of the cooperation between SidE and SidC effectors in the assembling of mixed ubiquitin chains will be crucial for a deeper mechanistic understanding of this process.

Another exciting direction for future research is the investigation of the potential interplay between the PR-ubiquitination machinery and other L. pneumophila effectors that target the host ubiquitin system. The L. pneumophila genome encodes a vast array of effectors with diverse functions, and it is likely that the SidE and SidC effectors work in concert with other effectors to orchestrate the complex host-pathogen interactions. For example, a very recent study highlights that LnaB of L.pneumophila reverses the PR-ubiquitin to ADPR-ubiquitin through the addition of AMP to the PR-ubiquitin[16]. It will be interesting to investigate the PR-moieties on the polyubiquitin chains created by cooperative reaction of Sdc and SidE are also accessible with LnaB.

From a broader perspective, the discovery of PR-ubiquitination as a mechanism to evade autophagy raises the intriguing possibility that similar strategies may be employed by other intracellular pathogens. It will be interesting to explore whether PR-ubiquitination or related modifications are utilized by other bacteria to subvert host defenses. Comparative studies across different pathogenic species could shed light on the evolutionary conservation and diversification of ubiquitin manipulation strategies.

In conclusion, the studies by Kotewicz et al. and Wan et al. represent a significant leap forward in our understanding of how L. pneumophila hijacks the host ubiquitin system to create a permissive replicative niche. The discovery of the cooperative action of SidE and SidC effectors in generating mixed ubiquitin chains and the identification of PR-ubiquitination as a mechanism to evade autophagy have unveiled new paradigms in host-pathogen interactions. These findings underscore the importance of considering the complex interplay between bacterial effectors and the potential for novel post-translational modifications in shaping the outcome of infections. As we continue to unravel the intricate strategies employed by pathogens to subvert host defenses, we will not only gain a deeper understanding of the pathogenesis of infectious diseases but also gain new insights into fundamental cellular processes. The exciting discoveries made by Kotewicz et al. and Wan et al. pave the way for future research that will further illuminate the molecular battles between pathogens and their hosts, ultimately informing the development of new therapeutic strategies to combat infections.

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S.K., M.C., H.J. wrote the manuscript, M.J. drew the figure. D.S. designed overall structure of manuscript and wrote the manuscript.

Competing interests

The authors declare no competing interests.

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