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COMMENT

Surviving the host–pathogen interaction: Legionella uses its SidE arsenal to mediate phosphoribosyl-linked ubiquitination of Golgi proteins inducing organelle fragmentation

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Host–pathogen interactions have been the subject of many research studies; as investigating the mechanisms by which pathogens invade, and replicates into, their hosts is necessary for the development of prevention and treatment strategies. Pathogens use a wide spectrum of molecular maneuvers to corrupt the host cellular processes and then utilize the resources for their own survival and propagation. *Legionella*, the causative agent of legionnaires' disease, is a gram-negative pleomorphic bacillus that causes pneumonia [1, 2]. These bacteria are able to replicate rapidly inside human cells including alveolar macrophages and other phagocytic cells and are known to survive by escaping the host's degradative defense systems [3]. During phagocytosis by macrophages, *Legionella* enters spacious vacuoles to form phagosomes that are quickly enveloped by endoplasmic reticulum (ER) features, which camouflage the phagosome signature and protect them from lysosomal degradation. A recent study reported by Dikic et al. shows that Legionella bacterium employs SidE effectors to promote phosphoribosyl-linked ubiquitination of Golgi proteins, leading to organelle disruption and altered host secretory pathway [4] (Fig. 1).

The canonical ubiquitination reaction consists of a covalent attachment of ubiquitin to internal lysine or N-terminal residues of target proteins through a three-step mechanism that typically involves three enzymes: E1 (ubiquitin-activating), E2 (ubiquitin-conjugating), and E3 (ubiquitin-ligase), resulting in a change of protein function or inducing protein degradation [5]. Given the importance of ubiquitination in the regulation of cellular processes and host cell immunity, it is not surprising that many pathogens have evolved survival strategies to hijack these host defense systems. Notably, despite the absence of ubiquitin in prokaryotes, bacterial pathogens have developed numerous genes involved in manipulating the host cell ubiquitin system. For instance, *Legionella* SidE family of effector proteins catalyze a non-conventional ubiquitination reaction, called phosphoribosyl-ubiquitination (PR-Ub), which involves NAD+, but not ATP [6]. SidE enzymes can directly conjugate a phosphoribosyl-ubiquitin to serine residues on their substrates via a phosphodiester bond without the need of E2 and E3 enzymes [6].

The study by Liu et al. now provides new insights into how SidE family of effectors exploits eukaryotic ubiquitination during *Legionella* infection [4]. Based on early studies showing that expression of SdeA protein, a member of SidE family of effectors, induces Golgi fragmentation [7], the authors first validated that SdeA overexpression in human cells colocalize with calnexin, a marker of ER, and GM130, a marker of the Golgi compartment. SdeA protein contains four distinct domains, the N-terminal deubiquitinase (DUB) domain, the phosphodiesterase (PDE) domain, the mono-ADP-ribosyltransferase (mART) domain, and the C-terminal coiled-coil (CC) domain. The PDE and mART are important for SdeA catalytic activity. However, the cellular functions associated with the CC domain remained incompletely understood. Interestingly, it was previously shown that CC domains found in other bacteria effectors such as *Salmonella* type III effectors are important for targeting to membranes [8]. Indeed, deletion of the CC domain caused a profound loss of SdeA colocalization with the ER and Golgi, suggesting its importance for subcellular localization.

In defining the functional significance of SdeA-mediated ubiquitination, the authors found that expression of SdeA, but not its catalytic-defective mutants, causes a dispersion of GM130 staining, suggesting that SdeA induces a disorganization of the Golgi structures. These results were corroborated by data obtained upon infection of human cells with *Legionella* strains. Indeed, host cells infected with SidE-defective strains showed normal Golgi structure compared to cells infected with wild-type Legionella. In addition, induction of high levels of PR-Ub, by ablating DupA/B deubiquitinases from *Legionella*, leads to a more pronounced Golgi fragmentation, consistent with previous studies [9]. Thus, disruption of Golgi structure by SidE effectors during pathogen infection is dependent on PR-Ub activity.

Next, the authors searched for new targets of SdeA in the Golgi. Using DupA as a bait, they identified several Golgi proteins including GRASP55 as potential targets of PR-Ub. GRASP55 has important functions in Golgi structure and maintenance [10], suggesting that PR-Ub of this protein could modulate Golgi integrity. Indeed, GRASP55 and another highly similar Golgi factor, GRASP65, were found to be modified by SdeA. Moreover, DupA removes PR-ubiquitination from GRASP55 and GRASP65, showing that these host Golgi proteins are specifically targeted by the PR-Ub machinery of *Legionella*. Moreover, the authors found that human cells infected with wild-type *Legionella* show GRASP55 and GRASP65 PR-ubiquitination, effects not observed with

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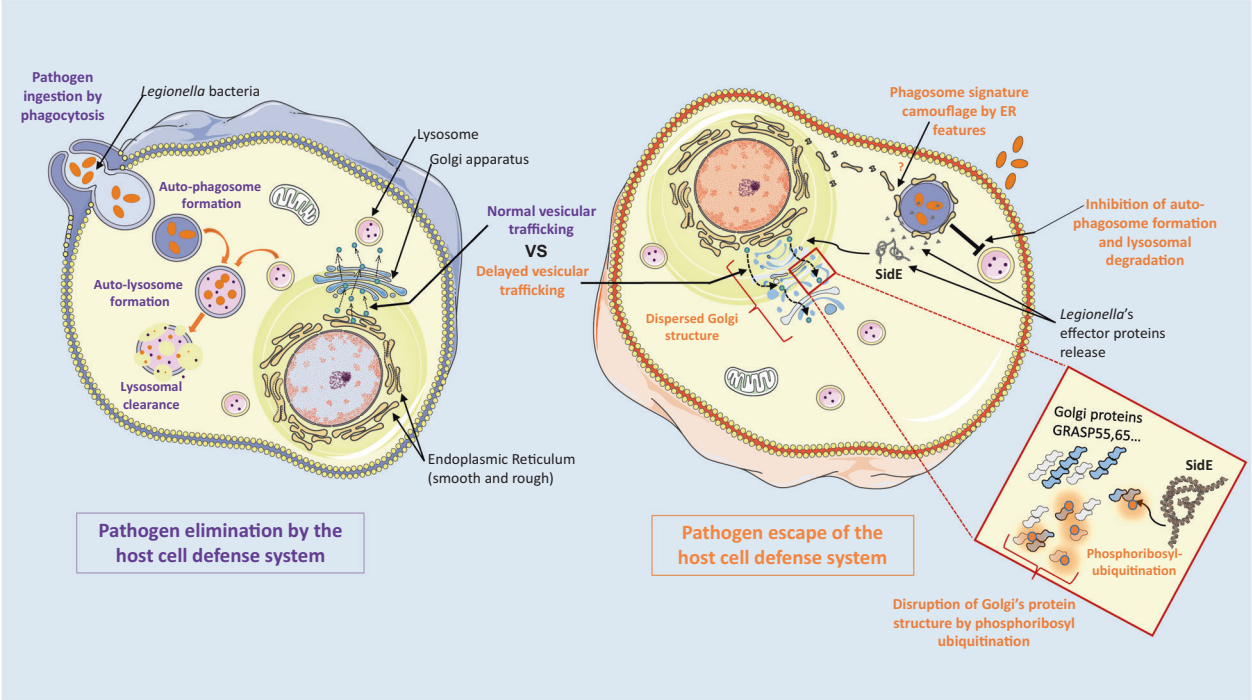

Fig. 1 *Legionella* employs SidE-mediated phosphoribosyl-ubiquitination to escape the host's cell defense mechanism. Left, bacteria pathogen elimination is achieved by the host cell proteolysis system. Bacteria are engulfed inside phagosomes and destroyed by the lysosome upon phagosome-lysosome fusion. Right, bacteria are able to wrap themselves with ER features avoiding recognition and degradation. In addition, *Legionella* SidE effector proteins hijack the host's cell ubiquitin system and catalyze phosphoribosyl-ubiquitination (PR-Ub), an atypic post-translational modification, of host cell proteins, thus conferring bacteria survival inside the host cells. Mechanistically, SdeA proteins ubiquitinate GRASP55 and GRAPS65, which are critical for the maintenance of Golgi structure, provoking organelle scattering. This in turn causes perturbation of the host cell secretory pathway.

SidE-defective bacteria strains. Importantly, PR-ubiquitination of GRASP55 and GRASP65 was further increased upon host cell infection with *Legionella* ΔDupA/B strain. Next, the authors used mass spectrometry and identified four serine residues in GRASP55 targeted by PR-Ub that were tested by mutagenesis for functional significance. Because SdeA protein does not have a highly specific motif of recognition, adjacent serines were also mutated to avoid redundant PR-ubiquitination. As expected, the seven serine mutants of GRASP55 present reduced PR-ubiquitination. Expressing GRASP55 PR-Ub defective mutant in cells expressing SdeA protein rescued Golgi scattering confirming that SdeA targets GRASP55 by PR-ubiquitination to provoke Golgi fragmentation.

Subsequently, the authors asked why would *Legionella* need to disrupt the Golgi during infection? Interestingly, *Chlamydia* infection is known to induce Golgi fragmentation to generate Golgi ministacks for bacterial inclusion and replication [11]. Thus, it was hypothesized that a similar mechanism could be used by *Legionella* to enhance the formation of *Legionella*-containing vacuole (LCV) by fusing with Golgi vesicles. However, staining of endogenous cis- and trans-Golgi proteins did not show colocalization with LCVs. The authors subsequently postulated that PR-ubiquitination of GRASP55 induces Golgi scattering and perturbates the cell secretory pathway, which could be beneficial for the growth of the pathogen. Indeed, using VSVG-GFP protein as a marker of protein trafficking in the Golgi, the authors found that infected cells with wild-type *Legionella* or ΔDupA/B mutant strain show decreased Golgi trafficking.

In summary, this study highlights how *Legionella* exploits the post-translational modification system of eukaryote cells to ensure its survival. The authors identified two novel substrates of PR-ubiquitination targeted by *Legionella*'s SdeA effector protein, GRASP55 and GRASP65. Their ubiquitination results in Golgi disruption and alteration of the secretory pathway of the host cell. In another perspective, Golgi structure dispersion upon *legionella* infection could also be important to target the host cell autophagy system in order to escape elimination. The Golgi complex regulates production and delivery of proteins and lipids needed for autophagy, in particular PI(4)P [12]. Thus, targeting Golgi structure and vesicular trafficking by *Legionella* effector proteins could be a key strategy to hijack degradative pathways such as xenophagy, a process by which host cells direct autophagy against their pathogen. Many studies underlined the implication of Golgi apparatus in the autophagosome–lysosome fusion for the xenophagy process [12–14]. For instance, it was reported that GRASP55 can also bridge the autophagosome to the lysosome to promote their fusion through interaction with LC3-II and LAMP2, respectively [15]. Therefore, potential inhibition of protein–protein interactions of GRASP55 by PR-ubiquitination upon *Legionella* infection could also disrupt their function in autophagosome–lysosome fusion.

In conclusion, this interesting study by Liu et al. [4] along with other studies give us novel insights and perspectives about how pathogens can exploit ubiquitin signaling to modulate cellular processes during infection.

REFERENCES

1. Fraser DW, Tsai TR, Orenstein W, Parkin WE, Beecham HJ, Sharrar RG, et al. Legionnaires' disease: description of an epidemic of pneumonia. N Engl J Med. 1977;297:1189–97.
2. McDade JE, Shepard CC, Fraser DW, Tsai TR, Redus MA, Dowdle WR. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N Engl J Med. 1977;297:1197–203.
3. Isberg RR, O'Connor TJ, Heidtman M. The *Legionella pneumophila* replication vacuole: making a cosy niche inside host cells. Nat Rev Microbiol. 2009;7:13–24.
4. Liu Y, Mukherjee R, Bonn F, Colby T, Matic I, Glogger M, et al. Serine-ubiquitination regulates Golgi morphology and the secretory pathway upon

Legionella infection. Cell Death Differ. 2021. https://doi.org/10.1038/s41418-021-00830-y. Online ahead of print.
5. Komander D, Rape M. The ubiquitin code. Annu Rev Biochem. 2012;81:203–29.
6. Qiu J, Sheedlo MJ, Yu K, Tan Y, Nakayasu ES, Das C, et al. Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. Nature. 2016;533:120–4.
7. Jeong KC, Sexton JA, Vogel JP. Spatiotemporal regulation of a *Legionella pneumophila* T4SS substrate by the metaeffector SidJ. PLoS Pathog. 2015;11:e1004695.
8. Knodler LA, Ibarra JA, Perez-Rueda E, Yip CK, Steele-Mortimer O. Coiled-coil domains enhance the membrane association of Salmonella type III effectors. Cell Microbiol. 2011;13:1497–517.
9. Wan M, Sulpizio AG, Akturk A, Beck WHJ, Lanz M, Faca VM, et al. Deubiquitination of phosphoribosyl-ubiquitin conjugates by phosphodiesterase-domain-containing Legionella effectors. Proc Natl Acad Sci USA. 2019;116:23518–26.
10. Rabouille C, Linstedt AD. GRASP: a multitasking tether. Front Cell Dev Biol. 2016;4:1.
11. Heuer D, Rejman Lipinski A, Machuy N, Karlas A, Wehrens A, Siedler F, et al. Chlamydia causes fragmentation of the Golgi compartment to ensure reproduction. Nature. 2009;457:731–5.
12. De Tito S, Hervas JH, van Vliet AR, Tooze SA. The Golgi as an assembly line to the autophagosome. Trends Biochem Sci. 2020;45:484–96.
13. Longatti A, Tooze SA. Vesicular trafficking and autophagosome formation. Cell Death Differ. 2009;16:956–65.
14. Geng J, Klionsky DJ. The Golgi as a potential membrane source for autophagy. Autophagy. 2010;6:950–1.
15. Zhang X, Wang L, Lak B, Li J, Jokitalo E, Wang Y. GRASP55 senses glucose deprivation through O-GlcNAcylation to promote autophagosome-lysosome fusion. Dev Cell. 2018;45:245–61. e246

AUTHOR CONTRIBUTIONS

OA and EBA discussed the study and established the ideas. OA wrote the initial draft of paper and made the figure. LM and EBA corrected and edited the manuscript. All authors reviewed and approved the manuscript before submission. Certain forms and shapes displayed in the figure were taken and modified from Servier Medical Art licensed under a Creative Common Attribution 3.0 Generic License (http://smart.servier.com/).

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The authors declare no competing interests.

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The manuscript was edified and written in agreement with established ethical standards.

ADDITIONAL INFORMATION

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