Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Emerging insights into bacterial deubiquitinases

Tomoko Kubori, Tomoe Kitao and Hiroki Nagai

Bacterial pathogens utilize eukaryotic cellular systems in various ways for their own benefits. To counteract host immune responses and survive in cells, bacteria modify host signaling pathways. For this aim, they have evolved virulence secretion systems. Bacteria-encoded effector proteins delivered via these secretion systems are the key players in bacterial pathogenesis. Ubiquitination is a post-translational modification that governs eukaryotic cellular systems. Recent studies have revealed that many bacterial effector proteins target the host ubiquitin system, often acting as ubiquitin-modulating enzymes such as ubiquitin ligases and deubiquitinases. Emerging lines of evidence have unveiled the diversity of bacterial deubiquitinases and have provided insights into the bacterial strategy to exploit the host ubiquitin system.

Address
Department of Microbiology, Graduate School of Medicine, Gifu University, 1-1 Yanagito, Gifu 501-1194, Japan

Corresponding author: Kubori, Tomoko (tkubori@gifu-u.ac.jp)

Current Opinion in Microbiology 2019, **47**:14–19

This review comes from a themed issue on **Host-pathogen interactions: bacteria**

Edited by **Karen M Ottemann** and **Linda J Kenney**

For a complete overview see the Issue and the Editorial

Available online 2nd November 2018

https://doi.org/10.1016/j.mib.2018.10.001

Introduction

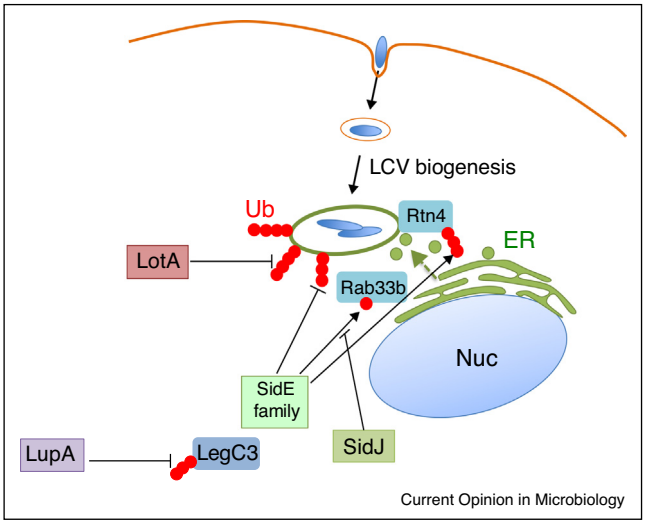
The vast majority of eukaryotic proteins are ubiquitinated during their lifespan, and their stability and/or activities are regulated via ubiquitination. Therefore, the ubiquitin system impacts many aspects of cellular processes. Ubiquitin is a small polypeptide of 76 amino acids that can be covalently conjugated to target proteins. Ubiquitination can occur as a result of a reaction cascade involving three universally conserved enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme) and E3 (ubiquitin ligase) [1].

There are many types of ubiquitination. Monoubiquitination is the conjugation of a single molecule of ubiquitin to a substrate protein, and it has been shown to regulate subcellular protein localization, conformation, activity and protein interactions [2]. Polyubiquitination is the binding of many ubiquitin molecules to the same target protein by the formation of ubiquitin chains. The polyubiquitination linkage type is determined based on the residues that are used to make the ubiquitin chains. Ubiquitin contains seven lysine residues (K6, K11, K27, K29, K33, K48 and K63) and an N-terminal methionine (M1) that can be conjugated to the C-terminus of a subsequent ubiquitin molecule. K48-linked polyubiquitin is known to mediate proteasomal degradation, while K63-linked polyubiquitin plays roles in cellular signaling, intracellular trafficking, DNA repair and so on [3]. With regard to other linkage types, the roles of polyubiquitin in cellular systems have not yet been fully determined.

Recently, a paradigm shift regarding ubiquitination occurred after the discovery of the SidE family of *Legionella pneumophila* effector proteins. These proteins are type IV secretion system (T4SS) substrates. The SidE family of proteins (SidE and its paralogues SdeA, SdeB and SdeC) bypass the requirement for E1 and E2 enzymes by catalyzing a noncanonical form of ubiquitination [4•,5]. The proteins have mono-adenosine diphosphate (ADP)-ribosyltransferase (mART) and phosphodiesterase (PDE) domains in a single polypeptide. Instead of the conventional adenosine triphosphate (ATP)-dependent reaction, the proteins catalyze nicotinamide adenine dinucleotide (NAD)-dependent ubiquitin transfer to substrate proteins such as Rab33b [4•] and reticulon 4 (Rtn4) [5] (Figure 1). The mART activity of the SidE family of proteins mediates the ADP-ribosylation of ubiquitin. The phosphodiester bond in the ADP-ribosylated ubiquitin is cleaved by the PDE activity to release adenosine monophosphate (AMP) and form phosphoribosylated ubiquitin (PR-Ub). The chain of reactions eventually results in unconventional serine ubiquitination of substrate proteins [6].

Deubiquitinases (DUBs) catalyze the removal of ubiquitin from substrate proteins. A DUB can either bind directly to the substrate protein and remove ubiquitin from it or, alternatively, it can recognize the ubiquitin chain and cleave the linkage between the ubiquitin molecules [7]. The linkage-specific cleavage implies that there are various ways of recognizing ubiquitin chains. About 100 DUBs are known to exist in humans [8]. Six structurally distinct DUB families have been described to date: (i) the ubiquitin-specific proteases (USPs), (ii) the ovarian tumor proteases (OTUs), (iii) the ubiquitin C-terminal hydrolases (UCHs), (iv) the Josephin family, (v) the motif interacting with ubiquitin (MIU)-containing novel DUB family (MINDYs) and (vi) JAB1/MPN/

Figure 1



Legionella DUBs.
After internalization into host cells, *L. pneumophila* establishes a replicative compartment, the so-called *Legionella*-containing vacuole (LCV), using endoplasmic reticulum (ER)-derived vesicles. Biogenesis of LCVs relies on ER-associated proteins, including reticulon 4 (Rtn4) and Rab GTPases. SidE family effectors (SidE, SdeA, SdeB and SdeC) can conjugate ubiquitin on Rtn4 and Rab33b by noncanonical ubiquitin ligase activity, facilitating ER membrane rearrangement in the vicinity of the LCV. The ubiquitin conjugates on Rab33b can be removed by the DUB activity of the cognate metaeffector protein SidJ. The DUB activity of the SidE family proteins contributes to remove ubiquitin chains from the LCVs. LotA also removes polyubiquitin chains from the LCVs. The DUB LupA can target another *Legionella* effector protein, LegC3, and remove ubiquitin chains from LegC3.

MOV34 (JAMM) metalloproteases (also known as MPN +) [7]. All but (vi) are cysteine proteases. Among them, OTUs are known to be highly linkage-specific [9]. The cysteine protease DUBs and ubiquitin-like (Ubl) proteases (ULPs) are classified as clan CA and CE peptidases (http://merops.sanger.ac.uk/index.htm) [10–12]. Clan CA contains families of peptidases that have structural similarity to papain. The families have the catalytic triad Cys/His/Asn (or Asp), which occur at this order in the primary sequence. Clan CE consists of cysteine peptidases with the catalytic triad His/Glu (or Asp)/Cys at this order.

Bacterial DUBs

There are many examples of bacterial ubiquitin ligases that are employed to modulate host cellular systems for bacterial benefits [13–16]. Similarly, accumulating lines of evidence indicate that DUBs are encoded by many bacterial species and play crucial roles after infection [12,17] (Table 1).

Salmonella and *Escherichia coli*

SseL is a type III secretion system (T3SS) effector protein of *Salmonella enterica serovar* Typhimurium. SseL was initially characterized as a bacterial protein similar to clan CE proteases [18]. Infection analyses showed that SseL was required for *Salmonella*-induced delayed cytotoxic effects on macrophages. SseL cleaves polyubiquitin chains, with a preference for K63-linked chains. The DUB activity of SseL was shown to contribute to evasion of selective autophagy by deubiquitinating T3SS-dependent ubiquitinated aggregates and aggresome-like induced structures (ALIS) [19]. Similar to SseL, ElaD of pathogenic *E. coli* is a clan CE cysteine protease. This protein was shown to have DUB activity *in vitro* [20] and to have a preference for K63-linked ubiquitin chains [21•].

Chlamydia

Cdu1 and Cdu2 (formerly termed ChlaDub1 and ChlaDub2, respectively) from *Chlamydia trachomatis* have domains with similarity to the catalytic domains of the clan CE proteases. NEDD8 (neural precursor cells developmental downregulated 8) is a Ubl modifier [22,23]. Both Cdu1 and Cdu2 were shown to have DUB activities *in vitro*, and they have dual specificity for ubiquitin and NEDD8 [21•,24]. The DUB activity of Cdu1 was shown to target IκBα (inhibitor of NFκB (nuclear factor κB)), resulting in inhibition of NFκB signaling and immune responses in order for the bacteria to survive in host cells [25]. Recently, it was reported that Cdu1 localizes on *Chlamydia*-containing vacuoles and stabilizes the apoptosis regulator, Mcl-1, using its DUB activity [26]. Another *Chlamydia* T3SS effector protein is the *Chlamydia pneumoniae* protein ChlaOTU, an OTU-like cysteine protease with DUB activity [27]. It was shown that this protein cleaves K48- and K63-linked polyubiquitin chains. Ubiquitin accumulation at *Chlamydia* entry sites was reduced by the catalytic activity of ChlaOTU. Interestingly, ChlaOTU possesses a domain that can interact with NDP52 (nuclear dot protein 52 kDa), an autophagy adaptor protein, but the implication of the interaction has not been fully clarified.

Burkholderia

Burkholderia mallei encodes a clan CA cysteine protease, TssM, the expression of which is transcriptionally co-regulated with a type VI secretion system (T6SS) gene cluster [28,29]. *In vitro* analysis showed that TssM has DUB activity and cleaves K48- and K63-linked ubiquitin chains [29]. TssM is implicated in the modulation of the host immune response upon *Burkholderia* infection [30]. TssM inhibits NFκB activation by interfering with TRAF6 (TNF (tumor necrosis factor) receptor-associated factor 6) and IκBα ubiquitination. Interestingly, the T6SS important for the invasion of macrophage was found to be dispensable for *B. pseudomallei* TssM secretion and expression in infected cells, while a T3SS was required for the expression of TssM in the cells [30]. Interplay between the two secretion systems might be involved in regulation of TssM function in infected cells.

Table 1

Bacterial DUBs

Bacteria	Protein	Cystein protease clan	Targets	Function	Reference(s)
Salmonella Typhimurium	SseL	Clan CE, Family C79	Unknown	Cytotoxicity Inhibition of selective autophagy	[18] [19]
Escherichia coli	ElaD	Clan CE, Family C79	Unknown	Unknown	[20,21•]
Chlamydia trachomatis			IkBa	Prevention of NFkB signaling	[24,25,21•]
	Cdu1	Clan CE, Family C48	Mcl-1	Stabilization of the apoptosis regulator Mcl-1	[26]
	Cdu2	Clan CE, Family C48	Unknown	Unknown	[24,21•]
Chlamydia pneumonia	ChlaOTU	Clan CA	Unknown	Reduction of ubiquitin accumulation at *Chlamydia* entry site	[27]
Burkholderia mallei	TssM	Clan CA, Family C19	TRAF6, IκBα	Inhibition of NFkB activation	[29,30]
Xanthomonas campestris	XopD	Clan CE, Family C48	Unknown	SUMO and ubiquitin isopeptidase	[32,33,21•]
Legionella pneumophila	SidE (Lpg0234)	Unknown	Unknown	Unknown	
	SdeA (Lpg2157	Unknown	Unknown	Regulation of ubiquitin associated with LCVs	[34]
	SdeB (Lpg2156)	Unknown	Unknown	Unknown	[34]
	SdeC (Lpg2153)	Unknown	Unknown	Unknown	[34]
	SidJ (Lpg2155)	Unknown	Rab33b	Phosphodiesterase to remove RP-Ub from substrates	[39••]
	SdjA (Lpg2508)	Unknown	Unknown	Unknown	[39••]
	LupA (Lpg1148)	Unknown	LegC3 (Lpg1701)	Rescue of yeast growth defect caused by LegC3 expression	[38••]
	LotA (Lpg2248)	Clan CA	Unknown	Regulation of ubiquitin associated with LCVs	[40•]

Xanthomonas

SUMO (small ubiquitin-like modifier) is another Ubl modifier [31]. The *Xanthomonas campestris* T3SS effector XopD was characterized as a cysteine protease that targets plant-specific sumoylated proteins [32,33]. This protein also belongs to the clan CE cysteine proteases [32]. Recent re-evaluation of XopD revealed that this protein is the first isopeptidase that has been found to be cross-reactive to ubiquitin and SUMO [21•]. Moreover, it was shown that XopD has a preference for K11-, K29- and K48-linked ubiquitin. Crystal structures showed that the unexpected cross-reactivity of XopD to ubiquitin and SUMO is attributable to an unstructured ubiquitin binding-region in this protein [21•].

Legionella

The noncanonical ubiquitin ligases, the SidE family of proteins in *L. pneumophila* described above, also possess DUB activity [34]. SdeA has structural similarity to Den1, a member of the ULP family of cysteine proteases. The DUB activity of SdeA has a preference for K63-linked ubiquitin chains. SdeA is able to recognize NEDD8 but not SUMO. The DUB activity of SdeA was found to be important for ubiquitin dynamics on *Legionella*-containing vacuoles (LCVs) (Figure 1).

Legionella encodes extraordinary number of effector proteins that are delivered via the T4SS [35]. Among the effector proteins, there is a class of effector proteins known as metaeffectors, which directly and/or functionally interacts with cognate effectors and regulate effector-modulated processes. The firstly identified metaeffector is *L. pneumophila* LubX which functions as an E3 ubiquitin ligase to mediate proteasomal degradation of another effector SidH [36]. Recently, genetic approaches taking advantage of yeast growth defects caused by ectopic expression of *L. pneumophila* effector proteins have been used to identify metaeffectors [37,38••]. For example, the ectopic expression of metaeffector SidJ suppressed the toxicity caused by expression of SdeA in yeast and mammalian cells [37]. Consistently with this observation, SidJ was discovered as a DUB having a

novel activity to cleave the phosphodiester bond between PR-Ub and substrates which ligation was mediated by the unprecedented SdeA ubiquitin ligase [39••]. SidJ is encoded at the same gene locus as the SidE family of proteins *sdeA*, *sdeB* and *sdeC* in *L. pneumophila*. The phosphodiesterase activity of SidJ was shown to be important for optimal intracellular growth of *L. pneumophila* and for efficient recruitment of endoplasmic reticulum (ER)-resident proteins to LCVs [39••]. SidJ also has canonical DUB activity, with a preference for K63-linkage, but it does not require catalytic cysteine residues [39••]. SdjA, a homolog of SidJ, did not exhibit detectable activity for proteins ubiquitinated by SdeA [39••]. However, the genetic interaction analysis utilizing yeast suggested that SdjA suppresses the activity of SidE, SdeB and SdeC more efficiently than SidJ [38••]. This raises the possibility that, similarly with SidJ, SdjA also has DUB activity for proteins ubiquitinated by the SidE family ubiquitin ligases. In this context, SidJ and presumably SdjA are metaeffectors for the SidE family of proteins.

The crystal structure of the *L. pneumophila* T4SS effector protein LupA revealed a eukaryotic ubiquitin protease (UBP)-like domain with a canonical cysteine protease triad [38••]. *In vitro* analysis confirmed that this protein is a DUB. A co-transfection experiment using human cells showed that the target of the DUB activity is another *L. pneumophila* effector, LegC3 [38••] (Figure 1). This finding provided another new insight into the existence of co-evolved effector pairs 'metaeffector and effector'.

Most recently, another *Legionella* DUB, LotA, was identified [40•]. LotA is distantly homologous to the OTU cysteine proteases and has two distinct catalytic cysteine residues. An *in vitro* analysis showed that LotA has DUB activity toward multiple ubiquitin linkage types. Upon infection, LotA functions to reduce ubiquitin decoration on LCVs in a catalytic cysteine-dependent manner. LotA possesses a phosphatidylinositol 3-phosphate (PI[3]P)-binding domain at its C-terminus. Its lipid-binding ability mediates LotA localization on the LCVs and is crucial for ubiquitin removal from LCVs (Figure 1). Interestingly, LotA preferentially cleaves polyubiquitin chains, rather than diubiquitins. The biological significance of DUB was demonstrated by a phenotypic analysis. When all the *sidE* family genes were disrupted, the intracellular growth of *L. pneumophila* was significantly reduced [34,37,41]. The growth defect was dependent on mART activity [4•,5] but not on DUB activity [34]. Disruption of *lotA*, in conjunction with the disruption of all *sidE* family genes, resulted in further reduction of intracellular growth [40•]. This suggests that the LotA DUB and the SidE family ubiquitin ligases functionally interact to optimize bacterial intracellular proliferation.

Another *Legionella* T4SS effector protein, RavZ, was shown to be a crucial player for preventing autophagy [42]. RavZ irreversibly deconjugated Atg8, a ubiquitin-like autophagy protein, from the lipid phosphatidylethanolamine (PE) on the early autophagosome. A *Legionella* and *Salmonella* co-infection experiment revealed that *Legionella* RavZ could reduce ubiquitin accumulated on *Salmonella*-containing vacuoles (SCVs) [43]. This finding raises the possibility that RavZ possesses DUB-like activity to deconjugate ubiquitin from unknown substrates on SCVs. Interestingly RavZ did not have a significant impact on the ubiquitin on LCVs. This implies that *Legionella* has a strategy to antagonize the potential RavZ activity toward LCVs.

Conclusions

Accumulating lines of evidence indicate that many pathogenic bacteria possess proteins with DUB activity. In fact, there are several other uncharacterized bacterial DUBs that were not covered in this opinion, including *Shigella flexneri* ShiCE and *Rickettsia bellii* RickCE [21•]. The known bacterial DUBs are likely to be just the tip of the iceberg, and a large array of unidentified DUBs may be present in pathogenic bacteria. One important role of the bacterial DUBs is antagonizing ubiquitin-mediated host cellular signaling pathways. This includes stabilization of cellular proteins that are destined for degradation by the ubiquitin-proteasome system. Recent studies on bacterial ubiquitin ligases and DUBs have been drastically expanding our understanding of how bacterial pathogens manipulate the host ubiquitin system. Studies of *Legionella* effectors have provided good examples of the functional interplay between bacterial effector proteins, known as metaeffector-effector relationships [36,38••,44,45]. Regarding deubiquitination effectors, the following relationships can be involved: First a bacterial ubiquitin ligase and a bacterial DUB that have the same cellular target (e.g. the target of SdeA and SidJ is Rab33b) and second a bacterial DUB that targets another bacterial protein (e.g. LupA targets LegC3). It is anticipated that some bacterial ubiquitin ligases have cognate bacterial DUBs. Future analyses of bacterial strategies to exploit the host ubiquitin system will provide insight into the orchestrated functional network of effector and host proteins.

Conflict of interest statement

Nothing declared.

Acknowledgements

Research in Nagai laboratory was supported by MEXT KAKENHI grant 15H01322 (to TKu) and by JSPS KAKENHI grants 26670212 (to HN), 16H05189, 16K14724 (to TKu), 18K15145, 17J40044 (to TKi). TKi is a recipient of JSPS research fellowship (RPD).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hershko A, Ciechanover A: **The ubiquitin system**. *Annu Rev Biochem* 1998, **67**:425-479.

2. Haglund K, Dikic I: **Ubiquitylation and cell signaling**. *EMBO J* 2005, **24**:3353-3359.

3. Komander D, Rape M: **The Ubiquitin code**. *Annu Rev Biochem* 2012, **81**:203-229.

4. • Qiu J, Sheedlo MJ, Yu K, Tan Y, Nakayasu ES, Das C, Liu X, Luo ZQ: **Ubiquitination independent of E1 and E2 enzymes by bacterial effectors**. *Nature* 2016, **533**:120-124.

This study provided the first description of the non-canonical all-in-one type of ubiquitin ligases that do not require E1, E2 and ATP. The finding prompted further research that led to greater understanding of chemical reactions involving non-canonical ubiquitin ligases.

5. Kotewicz KM, Ramabhadran V, Sjoblom N, Vogel JP, Haenssler E, Zhang M, Behringer J, Scheck RA, Isberg RR: **A single *Legionella* effector catalyzes a multistep ubiquitination pathway to rearrange tubular endoplasmic reticulum for replication**. *Cell Host Microbe* 2017, **21**:169-181.

6. Bhogaraju S, Kalayil S, Liu Y, Bonn F, Colby T, Matic I, Dikic I: **Phosphoribosylation of ubiquitin promotes serine ubiquitination and impairs conventional ubiquitination**. *Cell* 2016, **167**:1636-1649.e13.

7. Mevissen TET, Komander D: **Mechanisms of deubiquitinase specificity and regulation**. *Annu Rev Biochem* 2017, **86**:159-192.

8. Clague MJ, Barsukov I, Coulson JM, Liu H, Rigden DJ, Urbe S: **Deubiquitylases from genes to organism**. *Physiol Rev* 2013, **93**:1289-1315.

9. Mevissen TET, Hospenthal MK, Geurink PP, Elliott PR, Akutsu M, Arnaudo N, Ekkebus R, Kulathu Y, Wauer T, El Oualid F *et al.*: **OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis**. *Cell* 2013, **154**:169-184.

10. Barrett AJ, Rawlings ND: **Families and clans of serines peptidases**. *Perspective* 1996, **6**:1-11.

11. Rawlings ND, Barrett AJ, Thomas PD, Huang X, Bateman A, Finn RD: **The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database**. *Nucleic Acids Res* 2018, **46**:D624-D632.

12. Rytkönen A, Holden DW: **Bacterial interference of ubiquitination and deubiquitination**. *Cell Host Microbe* 2007, **1**:13-22.

13. Ashida H, Kim M, Sasakawa C: **Exploitation of the host ubiquitin system by human bacterial pathogens**. *Nat Rev Microbiol* 2014, **12**:399-413.

14. Maculins T, Fiskin E, Bhogaraju S, Dikic I: **Bacteria-host relationship: Ubiquitin ligases as weapons of invasion**. *Cell Res* 2016, **26**:499-510.

15. Lin Y-H, Machner MP: **Exploitation of the host cell ubiquitin machinery by microbial effector proteins**. *J Cell Sci* 2017, **130**:1985-1996.

16. Ashida H, Sasakawa C: **Bacterial E3 ligase effectors exploit host ubiquitin systems**. *Curr Opin Microbiol* 2017, **35**:16-22.

17. Zhou Y, Zhu Y: **Diversity of bacterial manipulation of the host ubiquitin pathways**. *Cell Microbiol* 2015, **17**:26-34.

18. Rytkonen A, Poh J, Garmendia J, Boyle C, Thompson A, Liu M, Freemont P, Hinton JCD, Holden DW: **SseL, a *Salmonella* deubiquitinase required for macrophage killing and virulence**. *Proc Natl Acad Sci* 2007, **104**:3502-3507.

19. Mesquita FS, Thomas M, Sachse M, Santos AJM, Figueira R, Holden DW: **The *Salmonella* deubiquitinase SseL inhibits selective autophagy of cytosolic aggregates**. *PLoS Pathog* 2012, **8**:e1002743.

20. Catic A, Misaghi S, Korbel GA, Ploegh HL: **ElaD, a deubiquitinating protease expressed by *E. coli***. *PLoS One* 2007, **2**:1-8.

21. • Pruneda JN, Durkin CH, Geurink PP, Ovaa H, Santhanam B, Holden DW, Komander D: **The molecular basis for ubiquitin and ubiquitin-like specificities in bacterial effector proteases**. *Mol Cell* 2016, **63**:261-276.

This study contains comprehensive analyses of bacterial clan CE proteases. Structural and functional analyses of newly identified as well as known bacterial DUBs, including XopD and SseL, provide insights into CE clan relationships across kingdoms and substrate specificity.

22. Rabut G, Peter M: **Function and regulation of protein neddylation. "Protein modifications: beyond the usual suspects" review series**. *EMBO Rep* 2008, **9**:969-976.

23. van der Veen AG, Ploegh HL: **Ubiquitin-like proteins**. *Annu Rev Biochem* 2012, **81**:323-357.

24. Misaghi S, Balsara ZR, Catic A, Spooner E, Ploegh HL, Starnbach MN: ***Chlamydia trachomatis*-derived deubiquitinating enzymes in mammalian cells during infection**. *Mol Microbiol* 2006, **61**:142-150.

25. Le Negrate G, Krieg A, Faustin B, Loeffler M, Godzik A, Krajewski S, Reed JC: **Chla Dub1 of *Chlamydia trachomatis* suppresses NF-κB activation and inhibits IκBα ubiquitination and degradation**. *Cell Microbiol* 2008, **10**:1879-1892.

26. Fischer A, Harrison KS, Ramirez Y, Auer D, Chowdhury SR, Prusty BK, Sauer F, Dimond Z, Kisker C, Hefty PS *et al.*: ***Chlamydia trachomatis*-containing vacuole serves as deubiquitination platform to stabilize Mcl-1 and to interfere with host defense**. *Elife* 2017, **6**:1-27.

27. Furtado AR, Essid M, Perrinet S, Balañá ME, Yoder N, Dehoux P, Subtil A: **The chlamydial OTU domain-containing protein ChlaOTU is an early type III secretion effector targeting ubiquitin and NDP52**. *Cell Microbiol* 2013, **15**:2064-2079.

28. Schell MA, Ulrich RL, Ribot WJ, Brueggemann EE, Hines HB, Chen D, Lipscomb L, Kim HS, Mrázek J, Nierman WC *et al.*: **Type VI secretion is a major virulence determinant in *Burkholderia mallei***. *Mol Microbiol* 2007, **64**:1466-1485.

29. Shanks J, Burtnick MN, Brett PJ, Waag DM, Spurgers KB, Ribot WJ, Schell MA, Panchal RG, Gherardini FC, Wilkinson KD *et al.*: ***Burkholderia mallei* tssM encodes a putative deubiquitinase that is secreted and expressed inside infected RAW 264.7 murine macrophagest**. *Infect Immun* 2009, **77**:1636-1648.

30. Tan KS, Chen Y, Lim YC, Tan GYG, Liu Y, Lim YT, MacAry P, Gan YH: **Suppression of host innate immune response by *Burkholderia pseudomallei* through the virulence factor TssM**. *J Immunol* 2010, **184**:5160-5171.

31. Geiss-Friedlander R, Melchior F: **Concepts in sumoylation: A decade on**. *Nat Rev Mol Cell Biol* 2007, **8**:947-956.

32. Hotson A, Chosed R, Shu H, Orth K, Mudgett MB: ***Xanthomonas* type III effector XopD targets SUMO-conjugated proteins in planta**. *Mol Microbiol* 2003, **50**:377-389.

33. Chosed R, Tomchick DR, Brautigam CA, Mukherjee S, Negi VS, Machius M, Orth K: **Structural analysis of *Xanthomonas* XopD provides insights into substrate specificity of ubiquitin-like protein proteases**. *J Biol Chem* 2007, **282**:6773-6782.

34. Sheedlo MJ, Qiu J, Tan Y, Paul LN, Luo Z-Q, Das C: **Structural basis of substrate recognition by a bacterial deubiquitinase important for dynamics of phagosome ubiquitination**. *Proc Natl Acad Sci* 2015, **112**:15090-15095.

35. Qiu J, Z-Q Luo: **Legionella and Coxiella effectors: strength in diversity and activity**. *Nat Rev Microbiol* 2017, **15**:591-605.

36. Kubori T, Shinzawa N, Kanuka H, Nagai H: ***Legionella* metaeffector exploits host proteasome to temporally regulate cognate effector**. *PLoS Pathog* 2010, **6**:e1001216.

37. Jeong KC, Sexton JA, Vogel JP: **Spatiotemporal regulation of a *Legionella pneumophila* T4SS substrate by the metaeffector SidJ**. *PLoS Pathog* 2015, **11**:1-22.

38. •• Urbanus ML, Quaile AT, Stogios PJ, Morar M, Rao C, Di Leo R, Evdokimova E, Lam M, Oatway C, Cuff ME *et al.*: **Diverse mechanisms of metaeffector activity in an intracellular bacterial pathogen, *Legionella pneumophila***. *Mol Syst Biol* 2016, **12**:893.

Utilizing the yeast growth defect caused by the expression of*Legionella* effector proteins, systematic analyses of effector-effector functional interactions were conducted. Using both structural and functional analyses, this study provided metaeffector candidates, including the newly identified *Legionella* DUB, LupA.

39. •• Qiu J, Yu K, Fei X, Liu Y, Nakayasu ES, Piehowski PD, Shaw JB, Puvar K, Das C, Liu X *et al.*: **A unique deubiquitinase that deconjugates phosphoribosyl-linked protein ubiquitination**. *Cell Res* 2017, **27**:865-881.
This study identified an unusual type of DUB that supresses the SidE effector-mediated noncanonical ubiquitination. The*Legionella* effector SidJ can cleave the phosphodiester bond that links PR-Ub to the substrate. SidJ also has canonical DUB activity.

40. • Kubori T, Kitao T, Ando H, Nagai H: **LotA, a *Legionella deubiquitinase*, has dual catalytic activity and contributes to intracellular growth**. *Cell Microbiol* 2018, **20**:e12840.
This study identified a*Legionella* DUB evolutionarily related to the OTU proteases. The DUB activity of LotA reduces ubiquitin accumulation on *Legionella* vacuoles in infected host cells. In conjunction with SidE ubiquitin ligases, LotA can contribute to bacterial growth in the cells.

41. Bardill JP, Miller JL, Vogel JP: **IcmS-dependent translocation of SdeA into macrophages by the *Legionella pneumophila* type IV secretion system**. *Mol Microbiol* 2005, **56**:90-103.

42. Choy A, Dancourt J, Mugo B, O'Connor TJ, Isberg RR, Melia TJ, Roy CR: **The *Legionella* effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation**. *Science* 2012, **338**:1072-1076.

43. Kubori T, Bui XT, Hubber A, Nagai H: ***Legionella* RavZ plays a role in preventing ubiquitin recruitment to bacteria-containing vacuoles**. *Front Cell Infect Microbiol* 2017, **7**:1-9.

44. Shames SR, Liu L, Havey JC, Schofield WB, Goodman AL, Roy CR: **Multiple *Legionella pneumophila* effector virulence phenotypes revealed through high-throughput analysis of targeted mutant libraries**. *Proc Natl Acad Sci U S A* 2017, **114**: E10446-E10454.

45. Hilbi H, Nagai H, Kubori T, Roy CR: **Subversion of host membrane dynamics by the Legionella Dot/Icm type IV secretion system**. In *Type IV Secretion in Gram-Negative and Gram-Positive Bacteria.* Edited by Backert S, Grohmann E. Cham: Springer; 2017:221-242.