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Title: Ubiquitin-regulating effector proteins from *Legionella*

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19 **ABSTRACT**

20 Ubiquitin is relatively modest in size but involves almost entire cellular signaling pathways.
21 The primary role of ubiquitin is maintaining cellular protein homeostasis. Ubiquitination
22 regulates the fate of target proteins using the proteasome- or autophagy-mediated degradation
23 of ubiquitinated substrates, which can be either intracellular or foreign proteins from invading
24 pathogens. *Legionella*, a gram-negative intracellular pathogen, hinders the host-ubiquitin
25 system by translocating hundreds of effector proteins into the host cell's cytoplasm. In this
26 review, we describe the current understanding of ubiquitin machinery from *Legionella*. We
27 summarize structural and biochemical differences between the host-ubiquitin system and
28 ubiquitin-related effectors of *Legionella*. Some of these effectors act much like canonical host-
29 ubiquitin machinery, whereas others have distinctive structures and accomplish non-canonical
30 ubiquitination via novel biochemical mechanisms.

INTRODUCTION

Regulation of cellular activities relies on various post-translational modifications (PTM), such as phosphorylation, glycosylation, or acetylation (1-3). Unlike the aforementioned PTM that transfers chemical moieties to the targets, ubiquitination ligates ubiquitin protein to the substrates (4, 5). The covalent attachment of the C-terminal carboxyl group of ubiquitin to the amine group of lysine residues on a target protein is called monoubiquitination. Ubiquitin also forms different types of chains, called polyubiquitin, through the ubiquitination on an amino group of 7 lysine residues (K6, K11, K27, K29, K33, K48, K63) or the first methionine (M1) residue of ubiquitin. The diverse conformational complexity of polyubiquitin chains gives cells abilities to differentiate and regulate various cellular processes. M1-linked linear polyubiquitin chain participates in inflammation and immune response. In the NF-kappaB (NF-kB) pathway, linear ubiquitin chain assembly complex (LUBAC) modifies NEMO, a subunit of inhibitor of kB (IkB) kinase (IKK) with M1 linkage that triggers phosphorylation of IkB (6-10). K6-linked polyubiquitin participates in DNA repair events and mitochondria stability (11-13), while K11- and K48- linked polyubiquitin is related to proteasomal degradation (14-17). K27-linked polyubiquitin controls DNA damage response and innate immune response. For instance, K27-linked polyubiquitination on histone 2A caused by RNF168 is related to DNA damage response (18). K29-linked polyubiquitination is associated with the Wnt signaling pathway, which regulates embryogenesis and tumorigenesis. K29-linked polyubiquitination on Axin, a scaffold protein in the Wnt signaling, disturbs interaction with LPR5/6 and inhibits the Wnt signaling (19, 20). It is known that K33 linkage participates in the regulation of T-cell antigen receptor and AMP-activated protein kinase-related protein kinase (21, 22). Together with K11- linked chain, K48 linkage plays a crucial role in the proteasome-dependent degradation pathway and ERAD pathway. Ubiquitin receptor on proteasome recognizes

homotypic K48 linkage chain on the substrates and guides the substrate to enter the proteasome (15, 23). K63 linkage participates in proteasome DNA damage repair and endosomal-lysosomal system (24, 25). Also, K63 linkage plays an essential role in immune signaling. K63-linked polyubiquitin chain acts as docking sites in immune pathway proteins. K63-linked polyubiquitin chain on MALT1 is related to I κ B α degradation and NF- κ B activation (26). Moreover, K63 linkage is associated with immunomodulatory function in T cells and NLR-mediated signaling, and STING signaling in viral infections (27, 28).

The canonical ubiquitination system consists of three stages of the catalytic cascade (1, 29). The ubiquitin-activating enzyme (E1) hydrolyzes ATP and catalyzes acyl adenylation of the ubiquitin. The AMP-Ub intermediate forms a thioester bond with the catalytic cysteine of the E1 (1, 30). Next, Ub on the E1 is transferred to the catalytic cysteine on the ubiquitin-conjugating enzyme (E2) (31, 32). Finally, E2 works with the ubiquitin ligation enzyme (E3) to attach Ub to the substrate. E3 ligases are divided into three major classes (HECT, RING, and U-box, RBR). Each of them has a unique mechanism to transfer ubiquitin to a substrate (33, 34). Most E3 ligases make an isopeptide bond between the carboxyl terminus of the ubiquitin and the amino group of Lys on the target protein or preceding Ub (1). All E3 ligases bind to E2~Ub thioesters and catalyze the transfer of ubiquitin from E2 to the substrate lysine. In particular, HECT (Homologous to E6-associated protein C terminus) type E3s have catalytic cysteine that accepts ubiquitin from E2 via the transthioesterification reaction (35). In contrast, RING (Really Interesting New Gene) type E3 ligases have no active site residues and mediate direct ubiquitin transfer from E2 to the substrates. RING E3 ligase contains a RING (or RING-like) domain responsible for binding to E2 and stimulating the ubiquitin transfer (36). The RING domain generally adopts a cross-brace structure with two structural Zn²⁺ ions. A related domain, known as a U-box, is similar in function and structural fold but has a

hydrophobic core instead of the structural metal ion (37). The RBR (**R**ING-**b**etween-**R**ING) has two RING domains but combines roles of both RING and HECT E3 ligase (38, 39). One of the RING domains of RBR binds to E2 like the canonical RING domain, while the other RING domain accepts ubiquitin in a similar way to the HECT E3 ligases. Similar to other PTM, ubiquitination is also a reversible process, and the ubiquitin on the target proteins is recycled by deubiquitinase (DUB), which cleaves the isopeptide bond between ubiquitin and the substrate. About 100 types of DUBs have been identified in humans, and they are classified into seven superfamilies. Six of them (USP, OTU, MJD, UCH, MINDY, and ZUFSP) are cysteine proteases, whereas JAMM belongs to a zinc-containing metalloprotease (40, 41).

Legionella pneumophila is a gram-negative pathogenic bacteria causing Legionnaires' disease (42). *Legionella* uses more than 300 effector proteins during the infection. These effectors disrupt the host cellular processes and create an ideal environment for bacterial survival and replication inside the host cell (43). Many of these effectors target the host-ubiquitin system. For instance, RavZ (**R**egion **a**llowing **y**acuole colocalization **Z**) hydrolyzes carboxyl-terminal glycine of Atg8 and blocks the host autophagy (44). The SidE (**S**ubstrates of **I**cm/**D**ot transporter **E**) family effector mediates non-canonical phosphoribosyl ubiquitination, which is required for ER- or Golgi compartment disruption (45, 46). In addition, RavD (**R**egion **a**llowing **y**acuole colocalization **D**) effector cleaves the M1 polyubiquitin chain to block the host NF- κ B signaling pathway (47). These results show that *Legionella* has developed various machinery that alters host ubiquitin signaling in canonical or non-canonical ways. Structural and biochemical studies on these effectors revealed that some effectors mimic the host proteins to participate in the host cellular processes, whereas others have completely different structures and mediate novel biochemical reactions. This review highlights structural and biochemical differences of *Legionella* proteins involved in the host ubiquitin system.

Canonical ubiquitin system-related *Legionella* effectors

1. LubX (*Legionella* U-box E3-ligase)

Some effectors have been demonstrated to interact directly with the host ubiquitination machinery using the U-box or F-box domain. Both LegU1 and LegAU13 are F-box proteins and integrate into mammalian SCF complexes (Skp, Cullin, F-box containing complex), and the LegU1 SCF complexes ubiquitinate host chaperone protein BAT3 (48, 49). Another *Legionella* effector, LubX (Lpg2830), is a U-box E3 ligase (50). Like the eukaryotic U-box, LubX has E2 specificity and interacts with eight E2s (UBE2D1, UBE2D3, UBE2D2, UBE2D4, UBE2E2, UBE2E3, UBE2W1, and UBE2L6) (51, 52). Intriguingly, LubX has two U-box motifs (U-box-1 and U-box-2). However, the LubX U-box-2 domain alone shows no E3 activity, whereas LubX U-box-1 is sufficient for interacting with E2s and catalyzes polyubiquitination. Structural analysis of LubX U-box-1 in a complex with human UBE2D2 reveals that most of the LubX U-box-1:UBE2D2 binding interface residues are not conserved in LubX U-box-2 (53). The overall structure of LubX:UBE2D2 is similar to the human UBE4B U-box:UBE2D3 complex (Figure 1. A) (54). However, residues at the binding interface differ from each other. Although both LubX U-box-1 and human UBE4B bind to the Ala-Pro-Ser hydrophobic patch on E2s, LubX U-box-1 makes additional hydrogen bond networks between Ile39-Arg5, Lys68-Ser91, and Arg75-Glu92. Importantly, these residues are not conserved in LubX U-Box-2. These structural analyses indicate that *Legionella* U-box distinguishes E2s through the sequence diversity on the U-box:E2 interface, just as the other U-box does. Functional roles of LubX E3 ligase is also reported. As a metaeffector, LubX target another substrate of the Dot/Icm system (52). LubX promotes human Cdc2-like kinase1 (Clk1)

degradation. Clk1 plays an important role in the modulation of host cellular processes. As a result, Clk1 contributes to maximum *Legionella* growth in mouse macrophages. In addition, LubX mediates proteasomal degradation of SidH (another effector protein of Dot/Icm system) (51, 52).

2. *Legionella* OTU-like deubiquitinases (Lot-DUBs)

Not only E3 ligases but also deubiquitinases are found in *Legionella*. Among seven DUB superfamilies, the OTU (Ovarian TUmour deubiquitinase) family explicitly shows linkage specificity toward polyubiquitin chains (55). For example, human OTUB1 preferentially cleaves the Lys48-linked chain, and human OTULIN cleaves the M1-linked chain (56-58). Interestingly, *Legionella* has several OTU-like DUBs (*Legionella* OTU-like DUBs, LotA, LotB, and LotC), and they also show different linkage specificities (59). Intriguingly, LotA has two OTU domains (LotA OTU1, LotA OTU2), whereas LotB and LotC have a single OTU domain (60, 61). The LotA OTU1 domain preferentially cleaves K6-linked di-ubiquitin, but the LotA OTU2 domain cleaves longer K48- and K63-linked chains (60). LotB cleaves K63-linked di- and polyubiquitin chains, and LotC cleaves K6-, K11-, K33-, K48-, and K63-linked di-ubiquitin (Di-Ub) chains(62-64).

The crystal structure of LotA OTU2, LotB, and LotC revealed that all three Lots consist of a catalytic domain (green) and an extended helical lobe (EHL) (yellow) (Figure 1. B). Because the EHL is not found in any other OTU family, EHL defines *Legionella* OTU DUBs as a unique OTU subfamily. Notably, the EHL domain provides a ubiquitin-binding site that interacts with ubiquitin through hydrophobic or electrostatic interactions in *Legionella* (60, 61, 63). LotA OTU2 binds to positively charged ubiquitin residues (R42, R72, R74) through

the acidic patch (D407, D410, D412) on the EHL. Indeed, the D410R mutant decreases the catalytic activity against K48- and K63-linked polyubiquitin. In addition, LotA OTU2 EHL binds to the hydrophobic I44 patch of distal ubiquitin through its hydrophobic surface around V398. LotB EHL also plays an important role in ubiquitin-binding. F143 and M144 of LotB EHL bind to the hydrophobic patch around F45 and A46 on the ubiquitin (60, 63). The EHL on LotC also provides a binding site for ubiquitin, and mutations on EHL (Y119R or Y149R) decrease the K48-linked ubiquitin cleavage activity of LotC. Therefore, the EHL domain, which is not found in the eukaryotic OTU-DUB, seems to be a critical determinant for providing the linkage specificity of *Legionella* OTUs (60). Though the exact functional role of all three Lots during infection is not clear, proteomics studies revealed their substrates, and further studies are awaited to reveal how Lots regulates these substrates (60).

3. RavD (Region allowing yacuole colocalization D)

RavD is another *Legionella* DUB that explicitly cleaves the M1-linked linear ubiquitin chain. The structure of RavD in complex with a linear di-ubiquitin chain revealed that di-ubiquitin uses the same binding interface as it binds to human M1-specific deubiquitinase OTULIN (Figure 1. C) (57). Given the crystal structure of RavD, it was suggested that RavD does not use a substrate-assisted catalytic activation mechanism as OTULIN does. However, recent studies indicate that both RavD and OTULIN use a common mechanism for recognizing the M1-ubiquitin chain (65, 66). Molecular dynamic simulations on the microsecond scale of RavD in complex with di-ubiquitin suggest that ubiquitin at the S1' site has flexibility and that the binding affinity is substantially weaker than at the S1 site. These results indicate that RavD also uses substrate-assisted catalysis as OTULIN does (47, 66).

Non-canonical ubiquitination-related *Legionella* effectors

1. Phosphoribosyl Ubiquitination

SidE family effectors (Substrates of Icm/Dot transporter E, SidEs) are multi-domain proteins. They consist of a deubiquitinase domain, PDE (phosphodiesterase) domain, mART (mono ADP ribosyl transferase) domain, and a coiled-coil domain. When SidEs are translocated in the host cell, they catalyze a unique ubiquitination process on host proteins, called phosphoribosyl ubiquitination (Figure 2. A) (67). The mART domain on SidEs transfers the ADP-ribose moiety from NAD⁺ (nicotinamide adenine dinucleotide) to Arg42 of ubiquitin and generates ADP-ribosylated ubiquitin (ADPR-Ub). The ADPR-Ub is then processed by the PDE domain (68). PDE releases the AMP moiety from ADPR-Ub and produces phosphoribosyl ubiquitin (PR-Ub) in the absence of a substrate. When there is a substrate during the ADPR-Ub processing, PR-Ub is transferred to a serine residue on substrate proteins. Like canonical ubiquitination, deubiquitinases specific to PR-Ub are also present (46, 69). DupA and DupB (**D**eubiquitinase for **P**R-ubiquitination A and B) are also *Legionella* effectors, and each has a phosphodiesterase domain. DupA/B cleaves and releases PR-Ub from serine residues in the substrate. *Legionella* also regulates SidEs by directly inhibiting the mART domain using the glutamylation by SidJ or SdjA (70-75). Most of the PR-ubiquitination machinery's structures are determined (46, 67, 68, 70, 71, 73, 76-79), and structures of both mART and PDE domains of SidEs differ from functionally similar enzymes found in other organisms. In humans, ADP-ribosylation is catalyzed by the poly-ADP-ribose polymerase (PARP) family (80). A comparison of SidE mART with human PARP1 and PARP3 clearly shows the overall structural differences (Figure 2. B). SidE mART is also different from the mART of other organisms

(HopU1 from *Pseudomonas syringae*, XopAI from *Xanthomonas axonopodis*) and shows unique conformations (77). The PDE domain of SidEs also shows unique structural features. Most of the phosphodiesterases found in humans have a cap lobe sitting on the top of the catalytic core. In contrast, the SidE PDE cap lobe is not located next to the catalytic core (Figure 2. C). Interestingly, both DupA and DupB share a structure similar to that of the SidE PDE domain and use identical catalytic sites while they mediate opposite reactions (PR-ubiquitin transfer and PR-ubiquitin cleavage) (Figure 2. D). It is shown that DupA/B have a strong affinity to Ub, ADPR-UB, and PR-ubiquitinated peptides. In contrast, the SdeA PDE domain has a weak affinity and doesn't integrate with PR-ubiquitinated peptides. High affinity to the PR-ubiquitinated substrate results in PR-deubiquitinase activity of DupA/B (46).

2. Transglutaminase induced ubiquitination

MavC (More regions allowing vacuole colocalization C, Lpg2147) and MvcA (MavC paralog A, Lpg2148) were initially discovered as ubiquitin Gln40 deamidase (81). Structural and biochemical studies reveal that MavC binds to an activated Ube2N~Ub conjugate and catalyzes an intramolecular transglutaminase reaction to produce a covalent bond between Gln40 of Ub and Lys92 of Ube2N (82, 83). In contrast, MvcA cleaves ubiquitin from Ube2N and reverses the MavC-mediated Ube2N ubiquitination (Figure 3. A) (84). Another *Legionella* effector, Lpg2149, binds to MavC and MvcA and blocks their function (81, 85). Structural comparison of MavC and MvcA to the Cif effector revealed that both MavC and MvcA have a large insertion domain on the top of the catalytic pocket (Figure 3. C) (81). An interesting point is that MavC and MvcA share a 50% sequence identity, use the same catalytic triad, and have similar structural folds while performing opposite reactions. Structural studies reveal that the substrate-recognition region on the MvcA defines its role as a deubiquitinase (84).

Conclusion

This mini-review summarized current understandings of the *Legionella* effector proteins that regulate host ubiquitin signaling. *Legionella* hijacks host ubiquitination systems by using effectors similar to canonical ubiquitination machinery or alters ubiquitination systems by introducing non-canonical ubiquitination systems, such as phosphoribosyl ubiquitination and transglutaminase-induced ubiquitination. Structural analysis of both canonical and non-canonical ubiquitin effectors revealed that these effectors have structures that distinguish them from host ubiquitination systems. *Legionella* OTU-like deubiquitinases (LotA/B/C), mART, PDE, and MavC/MvcA, have additional structural motifs or insertion regions that participate in the catalytic reaction or ubiquitin recognition. More importantly, it seems to be a general phenomenon in *Legionella* that a pair of similar effectors performs opposite reactions. SidE PDE transfers PR-ubiquitin, while DupA/B PDE removes PR-ubiquitin. MavC induces Ube2N ubiquitination, whereas MvcA reverses this reaction. They use identical catalytic residues but have different binding affinity or specificity for the substrates to define their roles.

Interestingly, the non-canonical ubiquitination mechanisms—phosphoribosyl ubiquitination and transglutaminase-induced ubiquitination—are found only in *Legionella*. Because *Legionella* is not the only pathogen that translocates effectors to the host cells, it might be possible to discover similar ubiquitin-regulating machinery from other pathogens. Further research is awaited to see whether the novel non-canonical ubiquitination system exists in another organism and to understand how these unique effectors are developed in *Legionella*. Yet, other human pathogens do not have such systems.

243

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248 **CONFLICTS OF INTEREST**

249 The authors declare no conflict of interest.

250

251

FIGURE LEGENDS

Figure 1. Structures of canonical ubiquitin system-related *Legionella* effectors

Structures of effectors that participate in or regulate canonical ubiquitin systems are presented. (A) Crystal structures of LubX (PDB:4WZ3), LubX U-Box-1 (PDB:4WZ3), LubX U-Box-2 (PDB:4XI1), and human UBE4B U box (PDB:3L1Z) are shown (from left to right, respectively). Superimposed structures of LubX U-box-1/2 and human UBE4M U-box are shown on the right. (B) Crystal structures of LotA OTU1(PDB:7F9X), LotB (PDB:6KS5), and LotC (PDB:6YK8) are presented. The conserved OTU catalytic core is depicted as green, and the extra helical lobe (EHL) is yellow. (C) Complex crystal structures of RavD:di-ubiquitin (PDB:6NJD) and hOTULIN:di-ubiquitin (PDB:3ZNV).

Figure 2. The overall scheme of **phosphoribosyl ubiquitination** and structures of key

effectors. (A) Phospho-ribosyl ubiquitination pathway. SidE family effectors (SidEs) consist of a PDE (phosphodiesterase) domain and a mART (mono ADP ribosyl transferase) domain, which can cause phospho-ribosyl ubiquitination. The mART domain on SidEs transfers the ADP-ribose moiety from NAD⁺ (nicotinamide adenine dinucleotide) to Arg42 of ubiquitin and generates ADP-ribosylated ubiquitin (ADPR-Ub). The PDE domain then processes the ADPR-Ub. PDE releases the AMP moiety from ADPR-Ub and produces phosphoribosyl ubiquitin (PR-Ub). PR-Ub is transferred to a serine residue on substrate proteins. DupA and DupB are phosphodiesterases that cleave and release PR-Ub from serine residues in the substrate. (B) Structural comparison between the SidE mART domain (PDB ID: 5ZQ5), PARP1 ART (PDB ID: 4DQY), and PARP3 ART domain (PDB ID:4GV4). (C) The structure of the SidE PDE domain (PDB ID:5ZQ5) and human Phosphodiesterase 4B (PDB ID:5OHJ) are compared. (D)

Structural comparison of DupA (PDB:6RYB), DupB (PDB:6B7M), and SidE PDE (PDB ID 5ZQ5).

Figure 3. The overall scheme of transglutaminase-mediated ubiquitination of Ube2N and structural comparison of MavC and MvcA (A) Transglutaminase-induced Ube2N ubiquitination mechanism. MavC (Lpg2147) binds to activated Ube2N-Ub. Intramolecular transglutamination generates an isopeptide bond between the Gln40 of ubiquitin and the Lys92 of Ube2N. MvcA (Lpg2148) specifically cleaves Ub from the Ube2N-Ub produced by MavC. (B) Structural comparison of MavC (PDB ID:5TSC), MvcA(PDB ID:6K11), and cif (PDB ID:4F8C). Catalytic cysteines are color-coded in red.

REFERENCES

1. Hershko A and Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* 67, 425-479
2. Mann M and Jensen ON (2003) Proteomic analysis of post-translational modifications. *Nat Biotechnol* 21, 255-261
3. Olsen JV and Mann M (2013) Status of large-scale analysis of post-translational modifications by mass spectrometry. *Mol Cell Proteomics* 12, 3444-3452
4. Goldstein G, Scheid M, Hammerling U, Schlesinger DH, Niall HD and Boyse EA (1975) Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells. *Proc Natl Acad Sci U S A* 72, 11-15
5. Yau R and Rape M (2016) The increasing complexity of the ubiquitin code. *Nat Cell Biol* 18, 579-586
6. Gerlach B, Cordier SM, Schmukle AC et al (2011) Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature* 471, 591-596
7. Ikeda F, Rahighi S, Wakatsuki S and Dikic I (2011) Selective binding of linear ubiquitin chains to NEMO in NF-kappaB activation. *Adv Exp Med Biol* 691, 107-114
8. Kirisako T, Kamei K, Murata S et al (2006) A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J* 25, 4877-4887
9. Tokunaga F, Sakata S, Saeki Y et al (2009) Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. *Nat Cell Biol* 11, 123-132
10. Walczak H, Iwai K and Dikic I (2012) Generation and physiological roles of linear ubiquitin chains. *BMC Biol* 10, 23
11. Morris JR and Solomon E (2004) BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum Mol Genet* 13, 807-817
12. Nishikawa H, Ooka S, Sato K et al (2004) Mass spectrometric and mutational analyses reveal Lys-6-linked polyubiquitin chains catalyzed by BRCA1-BARD1 ubiquitin ligase. *J Biol Chem* 279, 3916-3924
13. Ordureau A, Sarraf SA, Duda DM et al (2014) Quantitative proteomics reveal a feedforward mechanism for mitochondrial PARKIN translocation and ubiquitin chain synthesis. *Mol Cell* 56, 360-375
14. Braten O, Livneh I, Ziv T et al (2016) Numerous proteins with unique characteristics are degraded by the 26S proteasome following monoubiquitination. *Proc Natl Acad Sci U S A* 113, E4639-4647
15. Chau V, Tobias JW, Bachmair A et al (1989) A multiubiquitin chain is confined to specific lysine in a targeted short-lived protein. *Science* 243, 1576-1583
16. Gregori L, Poesch MS, Cousins G and Chau V (1990) A uniform isopeptide-linked multiubiquitin chain is sufficient to target substrate for degradation in ubiquitin-mediated proteolysis. *J Biol Chem* 265, 8354-8357
17. Shabek N, Herman-Bachinsky Y, Buchsbaum S et al (2012) The size of the proteasomal substrate determines whether its degradation will be mediated by mono- or polyubiquitylation. *Mol Cell* 48, 87-97

18. Gatti M, Pinato S, Maiolica A et al (2015) RNF168 promotes noncanonical K27 ubiquitination to signal DNA damage. *Cell Rep* 10, 226-238
19. Clevers H and Nusse R (2012) Wnt/beta-catenin signaling and disease. *Cell* 149, 1192-1205
20. Fei C, Li Z, Li C et al (2013) Smurf1-mediated Lys29-linked nonproteolytic polyubiquitination of axin negatively regulates Wnt/beta-catenin signaling. *Mol Cell Biol* 33, 4095-4105
21. Al-Hakim AK, Zagorska A, Chapman L, Deak M, Pegg M and Alessi DR (2008) Control of AMPK-related kinases by USP9X and atypical Lys(29)/Lys(33)-linked polyubiquitin chains. *Biochem J* 411, 249-260
22. Huang H, Jeon MS, Liao L et al (2010) K33-linked polyubiquitination of T cell receptor-zeta regulates proteolysis-independent T cell signaling. *Immunity* 33, 60-70
23. Thrower JS, Hoffman L, Rechsteiner M and Pickart CM (2000) Recognition of the polyubiquitin proteolytic signal. *EMBO J* 19, 94-102
24. Duncan LM, Piper S, Dodd RB et al (2006) Lysine-63-linked ubiquitination is required for endolysosomal degradation of class I molecules. *EMBO J* 25, 1635-1645
25. Hofmann RM and Pickart CM (1999) Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair. *Cell* 96, 645-653
26. Oeckinghaus A, Wegener E, Welteke V et al (2007) Malt1 ubiquitination triggers NF-kappaB signaling upon T-cell activation. *EMBO J* 26, 4634-4645
27. Ni X, Kou W, Gu J et al (2019) TRAF6 directs FOXP3 localization and facilitates regulatory T-cell function through K63-linked ubiquitination. *EMBO J* 38
28. Wang J, Yang S, Liu L, Wang H and Yang B (2017) HTLV-1 Tax impairs K63-linked ubiquitination of STING to evade host innate immunity. *Virus Res* 232, 13-21
29. Husnjak K and Dikic I (2012) Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. *Annu Rev Biochem* 81, 291-322
30. Schulman BA and Harper JW (2009) Ubiquitin-like protein activation by E1 enzymes: the apex for downstream signalling pathways. *Nat Rev Mol Cell Biol* 10, 319-331
31. Olsen SK and Lima CD (2013) Structure of a ubiquitin E1-E2 complex: insights to E1-E2 thioester transfer. *Mol Cell* 49, 884-896
32. Ye Y and Rape M (2009) Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol* 10, 755-764
33. Buetow L and Huang DT (2016) Structural insights into the catalysis and regulation of E3 ubiquitin ligases. *Nat Rev Mol Cell Biol* 17, 626-642
34. Deshaies RJ and Joazeiro CA (2009) RING domain E3 ubiquitin ligases. *Annu Rev Biochem* 78, 399-434
35. Berndsen CE and Wolberger C (2014) New insights into ubiquitin E3 ligase mechanism. *Nat Struct Mol Biol* 21, 301-307
36. Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S and Weissman AM (1999) RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc Natl Acad Sci U S A* 96, 11364-11369

37. Ohi MD, Vander Kooi CW, Rosenberg JA, Chazin WJ and Gould KL (2003) Structural insights into the U-box, a domain associated with multi-ubiquitination. *Nat Struct Biol* 10, 250-255
38. Marin I and Ferrus A (2002) Comparative genomics of the RBR family, including the Parkinson's disease-related gene parkin and the genes of the ariadne subfamily. *Mol Biol Evol* 19, 2039-2050
39. Spratt DE, Walden H and Shaw GS (2014) RBR E3 ubiquitin ligases: new structures, new insights, new questions. *Biochem J* 458, 421-437
40. Kwasna D, Abdul Rehman SA, Natarajan J et al (2018) Discovery and Characterization of ZUFSP/ZUP1, a Distinct Deubiquitinase Class Important for Genome Stability. *Mol Cell* 70, 150-164 e156
41. Reyes-Turcu FE, Ventii KH and Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem* 78, 363-397
42. Fields BS, Benson RF and Besser RE (2002) Legionella and Legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 15, 506-526
43. Ashida H, Kim M and Sasakawa C (2014) Exploitation of the host ubiquitin system by human bacterial pathogens. *Nat Rev Microbiol* 12, 399-413
44. Choy A, Dancourt J, Mugo B et al (2012) The Legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. *Science* 338, 1072-1076
45. Liu Y, Mukherjee R, Bonn F et al (2021) Serine-ubiquitination regulates Golgi morphology and the secretory pathway upon Legionella infection. *Cell Death Differ* 28, 2957-2969
46. Shin D, Mukherjee R, Liu Y et al (2020) Regulation of Phosphoribosyl-Linked Serine Ubiquitination by Deubiquitinases DupA and DupB. *Mol Cell* 77, 164-179 e166
47. Wan M, Wang X, Huang C et al (2019) A bacterial effector deubiquitinase specifically hydrolyses linear ubiquitin chains to inhibit host inflammatory signalling. *Nat Microbiol* 4, 1282-1293
48. Ensminger AW and Isberg RR (2010) E3 ubiquitin ligase activity and targeting of BAT3 by multiple Legionella pneumophila translocated substrates. *Infect Immun* 78, 3905-3919
49. Price CT, Al-Khodori S, Al-Quadani T et al (2009) Molecular mimicry by an F-box effector of Legionella pneumophila hijacks a conserved polyubiquitination machinery within macrophages and protozoa. *PLoS Pathog* 5, e1000704
50. Miyamoto K and Saito K (2018) Concise machinery for monitoring ubiquitination activities using novel artificial RING fingers. *Protein Sci* 27, 1354-1363
51. Kubori T, Hyakutake A and Nagai H (2008) Legionella translocates an E3 ubiquitin ligase that has multiple U-boxes with distinct functions. *Mol Microbiol* 67, 1307-1319
52. Kubori T, Shinzawa N, Kanuka H and Nagai H (2010) Legionella metaeffector exploits host proteasome to temporally regulate cognate effector. *PLoS Pathog* 6, e1001216
53. Quaile AT, Urbanus ML, Stogios PJ et al (2015) Molecular Characterization of LubX: Functional Divergence of the U-Box Fold by Legionella pneumophila. *Structure* 23, 1459-1469
54. Benirschke RC, Thompson JR, Nomine Y et al (2010) Molecular basis for the association of human E4B U box ubiquitin ligase with E2-conjugating enzymes UbcH5c and Ubc4. *Structure* 18, 955-965
55. Mevissen TET and Komander D (2017) Mechanisms of Deubiquitinase Specificity and Regulation. *Annu Rev Biochem* 86, 159-192

56. Edelmann MJ, Iphofer A, Akutsu M et al (2009) Structural basis and specificity of human otubain 1-mediated deubiquitination. *Biochem J* 418, 379-390
57. Keusekotten K, Elliott PR, Glockner L et al (2013) OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell* 153, 1312-1326
58. Mevissen TE, Hospenthal MK, Geurink PP et al (2013) OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. *Cell* 154, 169-184
59. Kitao T, Nagai H and Kubori T (2020) Divergence of Legionella Effectors Reversing Conventional and Unconventional Ubiquitination. *Front Cell Infect Microbiol* 10, 448
60. Shin D, Bhattacharya A, Cheng YL et al (2020) Bacterial OTU deubiquitinases regulate substrate ubiquitination upon Legionella infection. *Elife* 9
61. Takekawa N, Kubori T, Iwai T, Nagai H and Imada K (2022) Structural Basis of Ubiquitin Recognition by a Bacterial Ovarian Tumor Deubiquitinase LotA. *J Bacteriol* 204, e0037621
62. Kitao T, Taguchi K, Seto S et al (2020) Legionella Manipulates Non-canonical SNARE Pairing Using a Bacterial Deubiquitinase. *Cell Rep* 32, 108107
63. Ma K, Zhen X, Zhou B et al (2020) The bacterial deubiquitinase Ceg23 regulates the association of Lys-63-linked polyubiquitin molecules on the Legionella phagosome. *J Biol Chem* 295, 1646-1657
64. Schubert AF, Nguyen JV, Franklin TG et al (2020) Identification and characterization of diverse OTU deubiquitinases in bacteria. *EMBO J* 39, e105127
65. Schulze-Niemand E, Naumann M and Stein M (2021) The Activation and Selectivity of the Legionella RavD Deubiquitinase. *Frontiers in Molecular Biosciences* 8
66. Schulze-Niemand E, Naumann M and Stein M (2022) Substrate-assisted activation and selectivity of the bacterial RavD effector deubiquitinylase. *Proteins* 90, 947-958
67. Qiu J, Sheedlo MJ, Yu K et al (2016) Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. *Nature* 533, 120-124
68. Bhogaraju S, Kalayil S, Liu Y et al (2016) Phosphoribosylation of Ubiquitin Promotes Serine Ubiquitination and Impairs Conventional Ubiquitination. *Cell* 167, 1636-1649 e1613
69. Wan M, Sulpizio AG, Akturk A et al (2019) Deubiquitination of phosphoribosyl-ubiquitin conjugates by phosphodiesterase-domain-containing Legionella effectors. *Proc Natl Acad Sci U S A* 116, 23518-23526
70. Black MH, Osinski A, Gradowski M et al (2019) Bacterial pseudokinase catalyzes protein polyglutamylation to inhibit the SidE-family ubiquitin ligases. *Science* 364, 787-792
71. Bhogaraju S, Bonn F, Mukherjee R et al (2019) Inhibition of bacterial ubiquitin ligases by SidJ-calmodulin catalysed glutamylation. *Nature* 572, 382-386
72. Song L, Xie Y, Li C et al (2021) The Legionella Effector SdjA Is a Bifunctional Enzyme That Distinctly Regulates Phosphoribosyl Ubiquitination. *mBio* 12, e0231621
73. Gan N, Zhen X, Liu Y et al (2019) Regulation of phosphoribosyl ubiquitination by a calmodulin-dependent glutamylase. *Nature* 572, 387-391
74. Osinski A, Black MH, Pawlowski K, Chen Z, Li Y and Tagliabracci VS (2021) Structural and mechanistic basis for protein glutamylation by the kinase fold. *Mol Cell* 81, 4527-4539 e4528

75. Adams M, Sharma R, Colby T, Weis F, Matic I and Bhogaraju S (2021) Structural basis for protein glutamylation by the *Legionella* pseudokinase SidJ. *Nat Commun* 12, 6174
76. Akturk A, Wasilko DJ, Wu X et al (2018) Mechanism of phosphoribosyl-ubiquitination mediated by a single *Legionella* effector. *Nature* 557, 729-733
77. Dong Y, Mu Y, Xie Y et al (2018) Structural basis of ubiquitin modification by the *Legionella* effector SdeA. *Nature* 557, 674-678
78. Kalayil S, Bhogaraju S, Bonn F et al (2018) Insights into catalysis and function of phosphoribosyl-linked serine ubiquitination. *Nature* 557, 734-738
79. Wang Y, Shi M, Feng H et al (2018) Structural Insights into Non-canonical Ubiquitination Catalyzed by SidE. *Cell* 173, 1231-1243 e1216
80. Prokhorova E, Zobel F, Smith R et al (2021) Serine-linked PARP1 auto-modification controls PARP inhibitor response. *Nat Commun* 12, 4055
81. Valleau D, Quaile AT, Cui H et al (2018) Discovery of Ubiquitin Deamidases in the Pathogenic Arsenal of *Legionella pneumophila*. *Cell Rep* 23, 568-583
82. Puvar K, Iyer S, Fu J et al (2020) *Legionella* effector MavC targets the Ube2N~Ub conjugate for noncanonical ubiquitination. *Nat Commun* 11, 2365
83. Guan H, Fu J, Yu T et al (2020) Molecular Basis of Ubiquitination Catalyzed by the Bacterial Transglutaminase MavC. *Adv Sci (Weinh)* 7, 2000871
84. Gan N, Guan H, Huang Y et al (2020) *Legionella pneumophila* regulates the activity of UBE2N by deamidase-mediated deubiquitination. *EMBO J* 39, e102806
85. Mu Y, Wang Y, Huang Y et al (2020) Structural insights into the mechanism and inhibition of transglutaminase-induced ubiquitination by the *Legionella* effector MavC. *Nat Commun* 11, 1774

Figure 1. canonical ubiquitin system

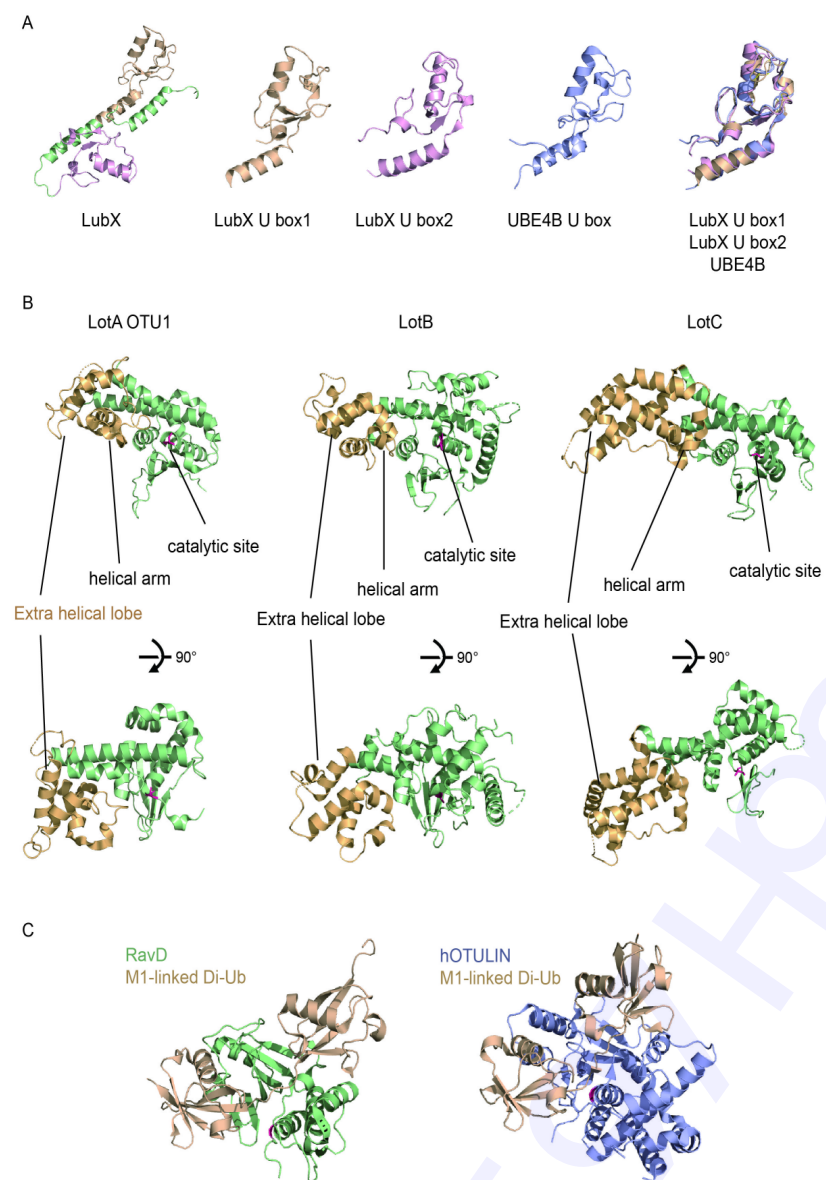


Fig. 1. Figure1

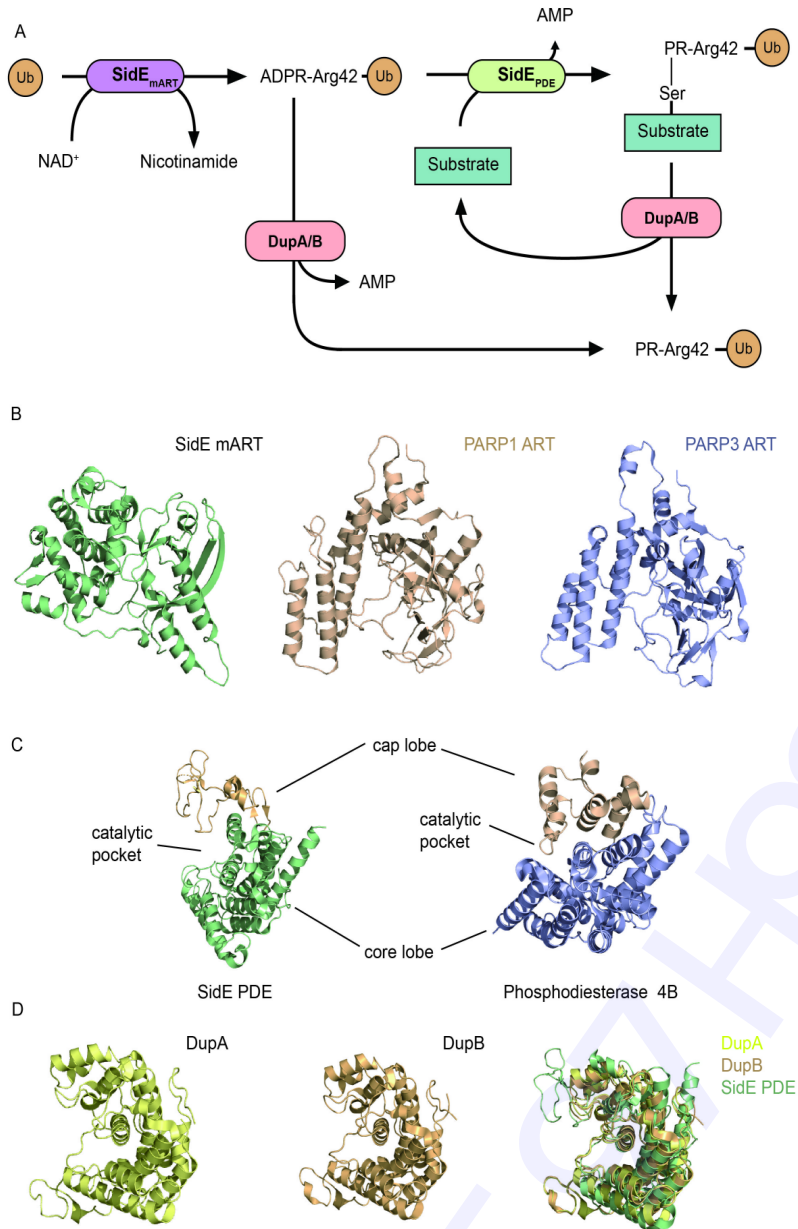
Figure 2. Phosphoribose ubiquitination

Figure 3. Transglutaminase induced ubiquitination

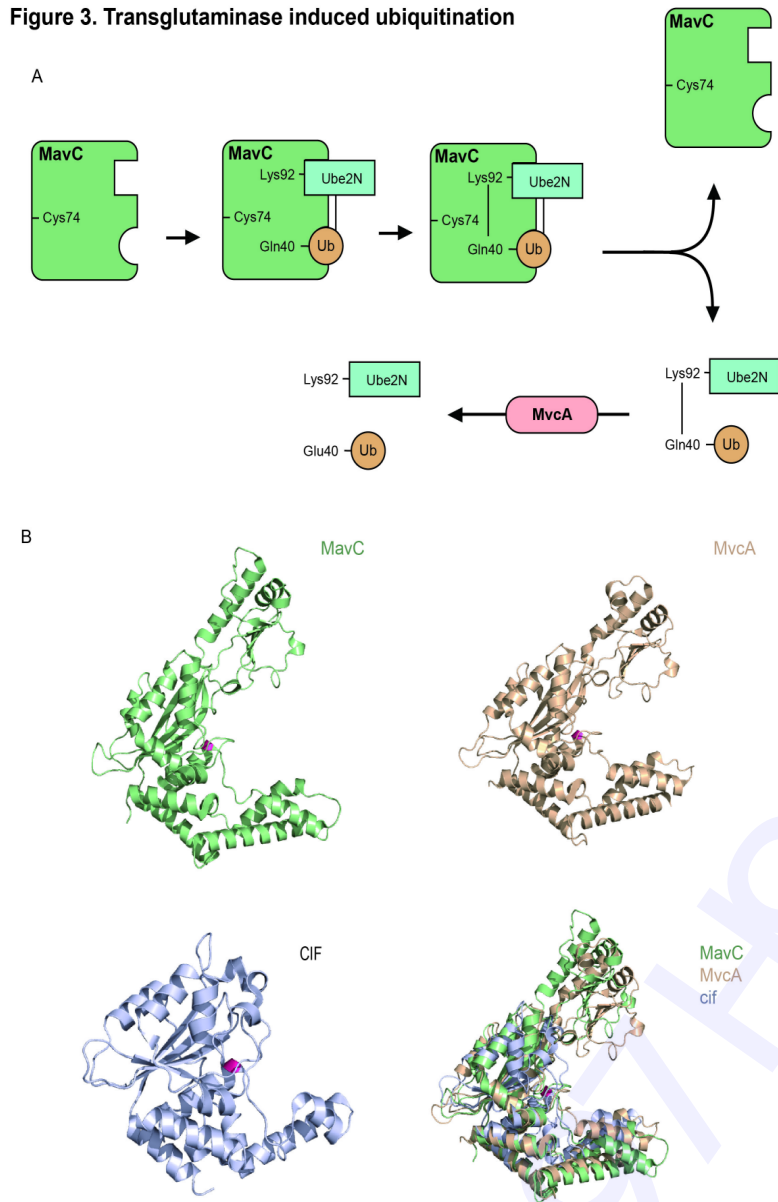


Fig. 3. Figure3