A bifunctional enzyme of *Legionella* that distinctly regulates phosphoribosyl ubiquitination of the SidE family effectors

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Legionella pneumophila is a Gram-negative intracellular pathogen that can replicate within freshwater amoeba and mammalian alveolar macrophages. In order to establish an intracellular niche permissive for its replication, *Legionella* translocates over 300 different effector proteins into the host cytosol via its Dot/Icm secretion system.[1] Among these, members of the SidE effector family (SidE, SdeA, SdeB, and SdeC) regulate multiple cellular processes by a unique phosphoribosyl (PR) ubiquitination mechanism that bypasses the canonical ubiquitination machinery,[1] and two phosphoglycosyl ubiquitin (PR-Ub)-specific deubiquitinases of *Legionella* (DupA and DupB) could cleave the PR-Ub induced by SidEs from PR-ubiquitinated substrates. The activity of the SidE family is regulated by a calmodulin (CaM)-dependent glutamylase effector of *Legionella*, named SidJ. Activated SidJ inhibits the adenosine diphosphate (ADP)-ribotransferase activity of SidEs by covalently attaching one or more glutamate moieties on the first glutamate residue of the ExE (where “x” represents any amino acid) element of the mono-ADP-ribosyl transferase (mART) domain that is essential for ubiquitin activation.[2,3] Interestingly, SdjA, a member of SidJ family in some *Legionella* strains, shares high level of sequence and structural identities to SidJ.[4] However, the function of SdjA is still unknown.

In a recent paper in *mBio*, Song *et al.* reveal that SdjA is a bifunctional enzyme that distinctly regulates PR-ubiquitination of members of the SidE family.[5] Firstly, Song *et al.* noted that SdjA was unable to alleviate the toxicity of SdeA, but effectively suppressed the yeast toxicity caused by SdeB or SdeC. Then, they examined the ubiquitination of Rab33b induced by the SidE family in a series of *L. pneumophila*-mutant strains and found that SdjA selectively inhibited the ubiquitin ligase activity of SdeB and SdeC, but not SidE and SdeA. Following subsequent biochemical experiments, the authors demonstrated that SdjA was another CaM-dependent glutamylase against SdeB and SdeC by catalyzing covalent attachment of a glutamate moiety to the first glutamate residue of the ExE element in the mART domains of SdeB and SdeC. Moreover, the authors unexpectedly found that SdjA removed SidJ-mediated glutamylation from Glu-SdeA and restored the Ub ligase activity of SdeA, and CaM was not required for the deglutamylase activity. The results reveal that SdjA is a bifunctional enzyme which inhibits the ubiquitin ligase activity of SdeB and SdeC by glutamylation and functions as a deglutamylase that reverses SidJ-induced glutamylation on SdeA (Figure 1).

The discovery of SdjA as a bifunctional enzyme raises several intriguing questions. First, how does SdjA selectively recognize and differently impact the activity of members of the SidE family? A recent study showed that the *N*-terminal domain (NTD) is involved in recognizing members of the SidE family.[6] Song *et al.* also noted that the NTD of SdjA was essential for its

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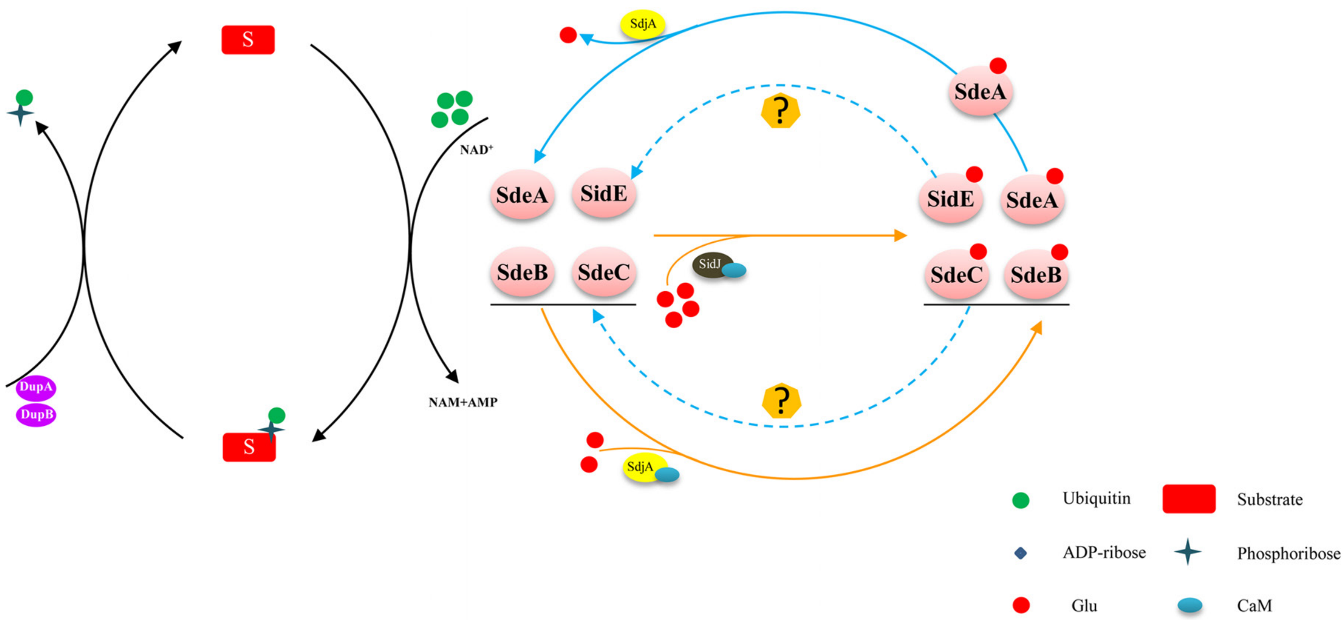

Figure 1: The PR-ubiquitination pathway and regulation of the SidE effector family. The SidE effector family (SidE, SdeA, SdeB, and SdeC) catalyzes a NAD-dependent PR ubiquitination. Two PR-Ub–specific deubiquitinases (DupA and DupB) cleave the PR-Ub from PR-ubiquitinated substrates. SidJ activated by CaM inhibits the ADP-ribosyltransferase activity of SidEs. In addition, SdjA inhibits the ubiquitin ligase activity of SdeB and SdeC by glutamylation and functions as a deglutamylase that reverses SidJ-induced glutamylation on SdeA. ADP: adenosine diphosphate; CaM: calmodulin; NAD: nicotinamide adenine dinucleotide; PR: phosphoribosyl; PR-Ub: phosphoribosyl ubiquitin.

glutamylation activity and a mutant of SdjA lacking the helix-turnhelix motif (HTH) and the NTD showed an indiscriminate deglutamylation activity toward all members of the SidE family. However, how SdjA recognizes members of the SidE family in the absence of NTD and the key motif or residues in SdjA for its deglutamylase activity are still under investigation. Second, CaM is essential for the glutamylation activity of SdjA, while it is dispensable for the deglutamylation activity, indicating their different catalytic mechanisms. Future structure-based analysis will likely provide insights into the exact catalytic mechanism for the peptidase activity of SdjA. Third, Song *et al.* noted that SdjA interferes with Rab33b ubiquitination induced only by SdeB or SdeC, but not by SidE. The result is not entirely consistent with Osinski *et al.*'s report, which revealed that SdjA glutamylated the active site Glu in SdeB, SdeC, and SidE and completely abolished Ub-ligase activity of SdeB, SdeC, and SidE *in vitro*.[6] Also, how the induced glutamylation on SdeB, SdeC, and SidE was revered remained to be investigated.

Remarkably, Song *et al.* revealed that an enzyme can catalyze two completely opposite biochemical reactions in regulating PR ubiquitination, which is a valuable contribution expanding our understanding of enzyme functions. The activity of SdjA and SidJ is reminiscent of MavC and MvcA, effectors of *L. pneumophila* which share 62% similarity in their primary sequences and function in regulation of the activity of the E2 enzyme UBE2N by opposite biochemical activities.[7-9] Due to these examples, it would not be surprising if other effectors of this pathogen co-opt the host ubiquitin network by opposite biochemical activities.

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Conflict of Interest

None declared.

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