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REVIEWS

Spotlight

Glutamylation of Bacterial Ubiquitin Ligases by a *Legionella* Pseudokinase

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Legionella pneumophila encodes a family of phosphoribosyl ubiquitination ligases (SidE) essential for the bacterium to establish successful infection. Four independent studies now show that the SidE family of ubiquitin ligases are regulated by a novel mechanism of glutamylation via a pseudokinase-like *Legionella* effector, SidJ, in an ATP- and calmodulin-dependent manner.

Legionella pneumophila is a Gram-negative intracellular pathogen of freshwater amoebas. However, inhalation of *Legionella*-contaminated aerosols can cause infection of human alveolar macrophages and lead to a severe form of pneumonia known as Legionnaires' disease. During infection, *Legionella* secretes more than 300 effector proteins via the Dot/Icm type IV secretion system to gain control over multiple host cellular processes, thereby converting the phagosome into the *Legionella*-containing vacuole (LCV), a specialized membrane-bound organelle amenable for its intracellular proliferation. One host cellular pathway subverted by *Legionella* is the ubiquitination pathway. So far, more than ten *Legionella* effectors have been identified as ubiquitin E3 ligases, including proteins containing the conserved F- or U-box domain and others akin to HECT-type E3 ligases.

In addition to these ubiquitin ligases, which utilize the canonical host ubiquitin-activating enzyme E1 and ubiquitin-conjugating enzyme E2, recent studies have identified the *Legionella* effector SidE and three homologs (SdeA, SdeB, and SdeC) as a family of unconventional ubiquitin ligases. These ligases act independently of ATP or E1 and E2 enzymes, but require a molecule of NAD^+ [1–3]. This novel type of ubiquitination, catalyzed by the SidE family ligases, is described as phosphoribosyl ubiquitination (PR-ubiquitination). In the reaction, SidE first consumes a NAD^+ molecule to generate an ADP-ribosylated ubiquitin (ADPR-Ub) by its mono-ADP-ribosyl transferase (mART) domain. Then, the phosphodiesterase (PDE) domain of SidE completes the PR-ubiquitination reaction by conjugating the phosphoribosyl-ubiquitin (PR-Ub) group from ADPR-Ub to a serine residue in targets. PR-ubiquitination is essential for *Legionella* optimal intracellular growth as the mutant strain carrying the deletion of all four SidE family members exhibited substantial growth defects in its amoeba host [4]. In the canonical ubiquitination pathway, ubiquitin can be cleaved from ubiquitinated substrates by deubiquitinases (DUBs). Similarly, PR-ubiquitination can also be reversed by *Legionella* PR-Ub-specific DUBs, which remove the PR-Ub moiety from PR-ubiquitinated substrates [5] (Figure 1). Furthermore, PR-ubiquitination is spatiotemporally regulated during *Legionella* infection. SidE proteins function at the LCV during early infection but are delocalized at a later stage. This has been attributed to the translocation of a *Legionella* metaeffector, SidJ [4]. SidJ is also able to suppress toxicity induced by overproduction of SidE family proteins in both yeast and mammalian cells [4,6], suggesting that SidJ may directly inhibit the PR-ubiquitination activity of SidE family ligases. However, the molecular mechanism underlying the regulation of SidE family ligases by SidJ had remained elusive.

Four recent independent studies now reveal that SidJ catalyzes an unusual type of post-translational modification to a key catalytic residue of the SidE family ligases in a calmodulin (CaM)-dependent manner [7–10]. These four papers describe either the X-ray crystal or the cryo- electron microscopy (EM) structure of SidJ in complex with CaM, with SidJ binding to CaM via a C terminal IQ motif-containing alpha helix. The structures also reveal that SidJ contains a bilobed kinase-like domain that retains a majority of the characteristic kinase catalytic motifs. Surprisingly, no phosphorylation events could be detected using conventional *in vitro* kinase assays with individually expressed and purified proteins. However, in a recent paper in *Science* [7], Black *et al.* noted that coexpression of SidJ, SdeA, and CaM in *Escherichia coli* resulted in a mobility shift of SdeA on SDS-PAGE. The authors further revealed that the total PR-ubiquitination signals were drastically reduced in cells expressing both SidJ and SdeA. In the *Nature* paper by Bhogaraju *et al.*, the authors found that coexpression of SidJ and SdeA in mammalian cells resulted in inhibition of ubiquitin modification by SdeA [8]. In another *Nature* paper, Gan *et al.* observed that purified SdeA from mammalian cells cotransfected with SdeA and SidJ failed to PR-ubiquitinate its substrate Rab33b [9]. Each of these findings suggests that SidJ is directly modifying SdeA. Indeed, mass spectrometry analyses performed by all four groups revealed that SdeA is polyglutamylated at the mART catalytic residue E860 in a SidJ-dependent manner. All four groups further demonstrated that, as a direct consequence of this modification, ADP-ribosylation of ubiquitin catalyzed by the SdeA mART domain is

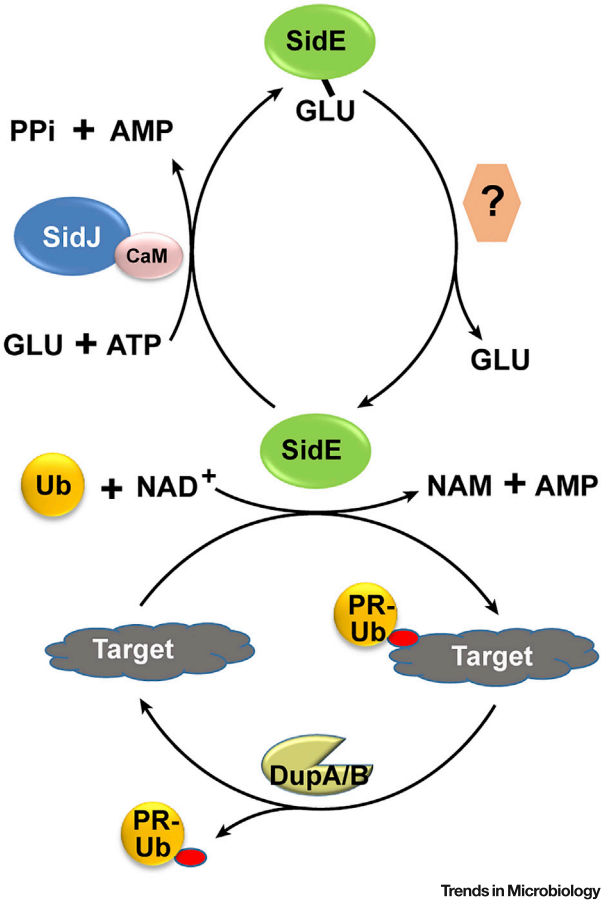

Figure 1. The Phosphoribosyl Ubiquitination (PR-ubiquitination) Pathway and Its Regulation by SidJ.

The SidE family effectors catalyze PR-ubiquitination using a molecule of NAD^+. DupA and DupB are two PR-Ub-specific deubiquitinases (DUBs) that cleave the phosphoribosyl-ubiquitin (PR-Ub) moiety from PR-ubiquitinated substrates. Upon activation by calmodulin (CaM), the metaeffector SidJ catalyzes the glutamylation of SidE family ligases and hence inhibits the PR-ubiquitination activity of SidE. An unknown effector may exist in *Legionella* to revive SidE family ligases by removing glutamate modification from the ligases. Abbreviations: GLU, glutamate; NAM, nicotinamide; PP_i, pyrophosphate.

obstructed, and thus the initiation of PR-ubiquitination is blocked (Figure 1).

The discovery of SidJ as a CaM-activated glutamylase raises several intriguing questions. First, how is CaM able to activate SidJ? In the paper by Sulpizio *et al.*, the authors propose that CaM-binding may stabilize a loop, which is equivalent to the activation loop in protein kinases, in an active conformation via a network of interactions involving the CaM N-lobe. Consistent with this idea, deletion of a N terminal peptide from SidJ, which directly mediates the contacts with the CaM N-lobe, substantially impairs SidJ activity [10]. Second, the crystal structures reported by Black *et al.* and Sulpizio *et al.* revealed a migrated nucleotide-binding pocket bound with an AMP molecule. This pocket was postulated by Black *et al.* as a catalytic site mediating the second step of the glutamylation reaction, while Sulpizio *et al.* showed evidence in support of an allosteric regulatory role for this pocket. To ascertain the exact mechanism of this migrated nucleotide-binding pocket requires further examination. Another important question is whether SidJ also modifies host targets during *Legionella* infection. Bhogaraju *et al.* found that glutamylation signals remained on the LCV when cells were infected by a *Legionella* mutant strain lacking all four *sidE* family members, indicating that SidJ may target additional proteins for glutamylation. Using a mass spectrometry approach, several host proteins were identified as potential glutamylation targets by SidJ [8]. Forthcoming studies of these host targets of SidJ will likely provide insights into the functions of SidJ beyond the regulation of SidE family enzymes.

Remarkably, these four recent papers on SidJ have unveiled an archetypal example of a pseudokinase-like bacterial effector protein catalyzing protein glutamylation in the context of PR-ubiquitination pathway regulation. Investigations are needed to elucidate how the preference for glutamate by SidJ is achieved and how the substrate specificity is determined by SidJ to selectively modify residue E860 of SdeA. Furthermore, based on multiple examples where *Legionella* employs effectors to regulate or reverse the activity of other effectors, it would not be surprising if this pathogen uses yet another effector to counteract SidJ by removing glutamate modification from SidE ligases and/or host proteins.

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Spotlight

The Respiratory Syncytial Virus Polymerase: A Multitasking Machine

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Respiratory syncytial virus (RSV) inflicts a significant toll on human life. An essential element of the virus is its polymerase, a complex capable of transcribing and replicating the viral genome. In an exciting new advance, Gilman *et al.* resolve the structure of this polymerase, providing valuable mechanistic insight into its activities.

RSV infects almost everyone worldwide by the age of two. It is the major cause of respiratory disease in infants, and it afflicts the elderly and immunocompromised [1]. As yet, there is no effective vaccine or antiviral therapy. The RSV polymerase is an essential component of the virus. With three enzymatic domains, it is a 'target-rich' complex for antiviral drug development, and has been subjected to mutations to generate live-attenuated vaccine candidates [2,3]. It is also a fascinating biological machine, capable of performing multiple activities to transcribe and replicate the RSV genome. Despite the importance of the RSV polymerase for translational research and basic biology, until now, its 3D structure remained elusive.

When RSV infects a cell, the viral genome is released from the virus particle into the cell cytoplasm. It is here that the genome undergoes two different processes: transcription to produce mRNAs required for viral protein synthesis, and replication to produce new viral genomes for packaging into virions. The RSV polymerase is capable of performing all enzymatic activities required for viral transcription and genome replication (reviewed in [2]). The genome is a single strand of negative-sense RNA coated along its length with multiple copies of the viral nucleoprotein. It contains ten genes, which are each transcribed into capped and polyadenylated mRNAs (Figure 1A). To transcribe the genome, the polymerase initiates at a promoter at the 3′ end of the genome and then moves towards the 5′ end, transiently displacing the nucleoprotein as it progresses. The polymerase is able to generate subgenomic mRNAs by responding to *cis*-acting signals that flank each gene. It initiates RNA synthesis at the beginning of a gene and cotranscriptionally adds a guanosine cap to the RNA 5′ end (Figure 1B), which it then methylates to produce a cap 1 structure. The polymerase then elongates the RNA until it reaches a gene end signal, where it adds a poly A sequence by repeatedly slipping on a uridine tract (Figure 1C). Following polyadenylation, the mRNA is released and the polymerase scans the genome to locate the start of the next gene and reinitiate RNA synthesis, transcribing the remaining genes. During replication, the nascent RNA is neither capped nor polyadenylated, but instead becomes associated with a growing chain of nucleoprotein as it is synthesized and is elongated to the end of the template. The replicative intermediate acts as a template for further rounds of genome synthesis. Remarkably, the core polymerase capable of all these activities consists of a complex of just two proteins, the large polymerase subunit (L) and phosphoprotein (P). The L subunit contains the RNA-dependent RNA polymerization, capping, and methyltransferase domains. These domains have properties that distinguish them from cellular enzymes. For example, it is likely that capping occurs by an RNA:GDP polyribonucleotidyltransferase rather than guanylyltransferase activity [4]. The P protein is required for efficient expression of L, and for contacting other viral proteins involved in RNA synthesis, such as nucleoprotein and a transcription elongation factor, M2-1 [5,6].

Now in a breakthrough achievement, Gilman and coworkers present the