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BACTERIAL PATHOGENESIS

Bacterial effectors skip a few steps

> SdeA catalyses substrate ubiquitylation independent of the E1 and E2 enzymes

During bacterial infection, host cells engage the ubiquitin system as part of their defence programme, but pathogens have evolved strategies to subvert or manipulate this role in host immunity. Protein ubiquitylation is achieved by the concerted action of activating enzymes (E1), conjugating enzymes (E2) and ligating enzymes (E3). Now, in a new study, Qiu *et al.* show that members of the SidE effector family in *Legionella pneumophila* directly ubiquitylate RAB small GTPases, independent of the E1 and E2 enzymes, during infection.

L. pneumophila uses numerous secreted effector proteins that manipulate host cell processes to support the establishment of the *Legionella*-containing vacuole (LCV), which provides an intracellular niche for replication. The Dot/Icm type IV secretion system delivers these effector proteins into the cytosolic compartment of the host cell. However, thus far, the roles of most Dot/Icm effectors have not been identified. Qiu *et al.* generated a *L. pneumophila* strain that lacks the Dot/Icm effector proteins from the SidE family, namely SdeA, SdeB, SdeC and SidE (Δ*sidE*) and found that this mutant had attenuated virulence. The expression of wild-type SdeA in the Δ*sidE* mutant strain restored its ability to grow in a protozoan host and its ability to recruit an endoplasmic reticulum (ER) marker to LCVs, which is a hallmark of infection with *L. pneumophila*. However, the expression of an SdeA mutant that has a mutation in a predicted mono-ADP-ribosyltransferase (mART) motif did not complement these defects in a Δ*sidE* mutant, which suggests that this motif is important for the function of the SidE protein family during bacterial infection.

Next, using mass spectrometry, the authors showed that SdeA induces the ubiquitylation of ER-associated RAB proteins and that substrate modification was dependent on the presence of the mART motif in SdeA. In addition, the authors found that SdeA-induced RAB ubiquitylation was required for the formation of the LCV, which indicates that the ubiquitylation of RAB proteins has a crucial role in intracellular bacterial growth. Furthermore, the overexpression of wild-type RAB33b restricted the generation of replicative vacuoles. In canonical ubiquitylation reactions, ubiquitin is activated by E1 in an ATP-dependent process and is delivered to E2 to form the E2–ubiquitin thioester; the ubiquitin moiety is then transferred from E2 to a substrate by the E3 ligase. Interestingly, the authors did not detect SdeA-mediated RAB33b ubiquitylation in a series of reactions that contained the E1 and E2 enzymes but they did in reactions that contained heat-treated cell lysates. These finding suggest that SdeA catalyses substrate ubiquitylation independent of the E1 and E2 enzymes but requires a heat-stable molecule that is present in cells. In agreement with this, reactions that contained ATP and Mg^{2+}, which are required for E1-dependent ubiquitin activation, did not lead to substrate modification by SdeA. By contrast, the addition of NAD (which is the donor of the ADP-ribose moiety transferred to the substrate by proteins that contain the mART motif) resulted in the ubiquitylation of RAB33b by SdeA and other SidE family members. The results suggest that NAD is sufficient for substrate ubiquitylation by these bacterial effectors. Finally, the authors provide evidence that SdeA directly activates ubiquitin by ADP-ribosylation and that the activated ubiquitin moiety is directly transferred to the substrate.

In summary, the findings from this study suggest that SdeA functions as an 'all-in-one' ubiquitin-conjugating enzyme that directly ADP-ribosylates ubiquitin and transfers activated ubiquitin to host proteins. Future work is required to determine how ubiquitylation of ER-associated RAB proteins promotes the formation of the LCV and thus pathogen survival.

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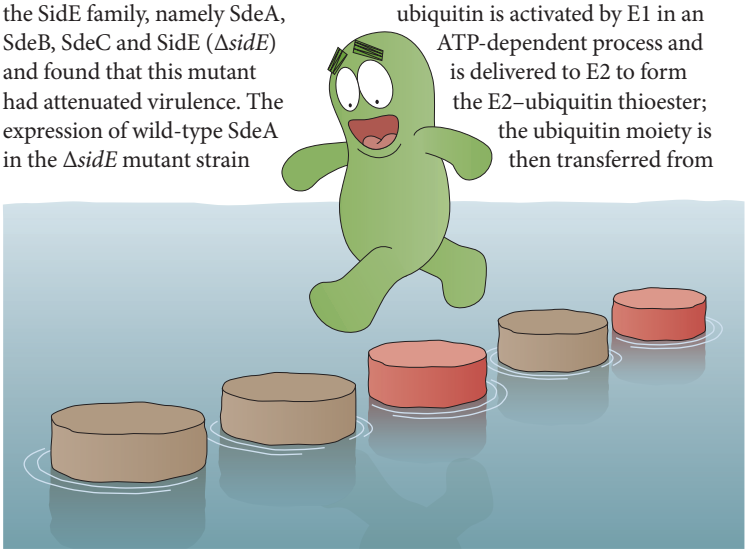

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ORIGINAL ARTICLE Qiu, J. *et al.* Ubiquitination independent of E1 and E2 enzymes by bacterial effectors. *Nature* http://dx.doi.org/10.1038/nature17657 (2016)

FURTHER READING Ashida, H., Kim, M. & Sasakawa, C. Exploitation of the host ubiquitin system by human bacterial pathogens. *Nat. Rev. Microbiol.* **12**, 399–413 (2014)