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Structure of a Shigella effector reveals a new class of ubiquitin ligases

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Abstract: Ubiquitination has an important role in almost every cellular process, including cell-cycle progression, endocytosis and trafficking, and immune-signal transduction. Ubiquitination involves a three-enzyme cascade including ubiquitin-activation enzyme E1, ubiquitin-conjugating enzyme E2 and ubiquitin ligase E3. It is recently recognized that bacterial pathogens have evolved effector proteins with ubiquitin E3 ligase activities through structural mimicking to interfere with the host's ubiquitination pathway. IpaH family including IpaH proteins from *Shigella flexneri*, is a large family of ubiquitin E3 ligases found exclusively in bacterial pathogens or symbionts. The IpaH family has the different N-terminal LRR domains and a conserved C-terminal domain, and shows no sequence homology to the two classes of eukaryotic E3 ligases: RING- and HECT-type E3s. The nature of this new pathogenic E3 ligase family and the underlying mechanism of ubiquitin transfer remain unknown. Here we report the 2.8 Å crystal structure of the *Shigella flexneri* effector IpaH3. The N-terminal LRR domain is structurally similar to *Yersinia pestis* YopM and potentially binds to substrates. The structure of the C-terminal E3 domain which has the full E3 ligase activity, differs from the typical RING- and HECT-type E3s. IpaH3 synthesizes a Lys48-linked ubiquitin chain, and the reaction requires noncovalent binding between ubiquitin and a specific E2, UbcH5. Cys363 within a conserved CXD motif acts as a nucleophile to catalyze ubiquitin transfer through a transthiolation reaction. The D365N mutant is devoid of E3 activities but turns into a potent ubiquitin-E2 thioesterase. Our analysis establishes a structurally and mechanistically distinct class of ubiquitin ligases found exclusively in pathogenic or symbiotic bacteria.

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