BACTERIAL PATHOGENESIS

A sweet interaction with death for EPEC

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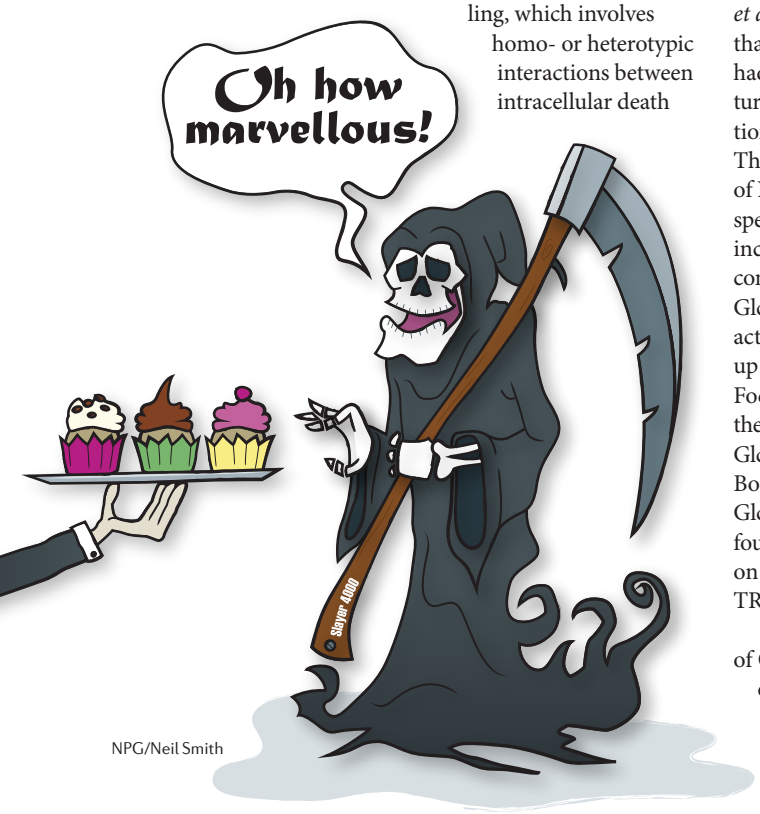
Two new papers in *Nature* reveal that the *Escherichia coli* effector protein NleB blocks host death receptor signalling by a novel *N*-acetylglucosamine (GlcNAc) transferase activity.

The attaching and effacing (A/E) pathogen enteropathogenic *E. coli* (EPEC) uses its type III secretion system to secrete effector proteins into host cells, subverting host cell functions to promote colonization. In this way, EPEC can modulate a range of host cell processes, including inhibiting NF-κB signalling and pro-inflammatory cytokine production. There are various pathways for NF-κB activation, one of which requires recognition of members of the tumour necrosis factor (TNF) family by TNF receptors (TNFRs). This initiates death receptor signalling, which involves homo- or heterotypic interactions between intracellular death domains in the receptors and death domains in the downstream adaptor proteins, including TRADD (TNFR1-associated death domain) and FADD (FAS-associated death domain). Death domain-containing proteins also regulate cell death by caspase 8-dependent apoptosis.



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Both groups were interested in the interaction between NleB, a conserved type III effector protein, and the host. Using a yeast two-hybrid screen of a cDNA library derived from HeLa cells together with co-immunoprecipitation assays, both groups pinpointed death domain-containing proteins as the host cell interacting partners for NleB, which was recently shown to have GlcNAc transferase activity in the EPEC-like pathogen *Citrobacter rodentium*. In their assays, Li, Zhang *et al.* picked up TRADD. They found that the NleB–TRADD interaction had no effect on TRADD stability or turnover, but did disrupt oligomerization of the TRADD death domain. The GlcNAc transferase activity of NleB was confirmed by mass spectrometry, which indicated a mass increase in TRADD of 203 daltons, consistent with the addition of a single GlcNAc moiety. In their protein interaction assays, Pearson *et al.* picked up multiple death domain proteins. Focusing on FADD, they confirmed the NleB-mediated addition of a single GlcNAc moiety by mass spectrometry. Both groups investigated the site of GlcNAc addition and, unexpectedly, found that this modification occurred on an Arg residue (Arg235 for TRADD, and Arg117 for FADD).

What are the consequences of GlcNAcylation of these death domain-containing adaptor proteins? Li, Zhang *et al.* found that death receptor signalling and apoptosis were inhibited in HeLa cells infected with EPEC expressing wild-type NleB, but not those expressing a catalytic-site mutant, and that a functional type III secretion system was required for inhibition. In caspase 8-dependent apoptosis, the FAS receptor is engaged by FAS ligand (FASL) and recruits FADD through homotypic death domain interactions. In turn, FADD recruits pro-caspase 8 into a death-inducing signalling complex (DISC), where it is activated. Using immunoblotting and immunofluorescence microscopy, Pearson *et al.* found that in host cells in which NleB was expressed ectopically, caspase 8 was not activated and cell death was inhibited. Moreover, infection of cells with EPEC inhibited FASL-induced DISC formation, and this required the GlcNAc transferase activity of NleB. Finally, both groups went on to show that the NleB-mediated GlcNAcylation inhibited death receptor signalling in a mouse model of EPEC infection.

Together, these papers identify EPEC NleB as the first known bacterial virulence factor to target death receptor signalling. Inhibition of host cell death in this way might prolong colonization. Both groups suggest that this novel virulence mechanism could be common in A/E pathogens.

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ORIGINAL RESEARCH PAPERS Li, S. *et al.* Pathogen blocks host death receptor signalling by arginine GlcNAcylation of death domains. *Nature* http://dx.doi.org/10.1038/nature12436 (2013) | Pearson, J. S. *et al.* A type III effector antagonises death receptor signalling during bacterial gut infection. *Nature* http://dx.doi.org/10.1038/nature12524 (2013)

FURTHER READING Gao, X. *et al.* NleB, a bacterial effector with glycosyltransferase activity, targets GAPDH function to inhibit NF-κB activation. *Cell Host Microbe* **13**, 87–99 (2013)