Bacterial pathogens use several strategies to infect host cells, one of which involves blocking host defenses. During infection, the bacterial effector proteins GtgA, GogA, PipA, and NleC are injected into host cells by the type III secretion system (T3SS), where they suppress the proinflammatory NF-κB signaling pathway to dampen immune responses. The authors demonstrate that these effectors bind NF-κB via their DNA-mimicking regions and uncover differences in effector sequences and structures explaining the individual specificities of these effectors for distinct NF-κB subunits.

Pathogenic Gram-negative bacteria such as Salmonella species and Escherichia coli use type III secretion systems (T3SSs) to inject host cells with effector proteins that suppress the host’s immune responses. One of the targets of some bacterial effectors is the protein complex NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells), which activates transcription of genes involved in immunity, inflammation, and cell survival in many mammalian species, including humans (1). Specifically, NF-κB is targeted by the zinc metalloprotease family of bacterial effector proteins that contain the highly conserved HEXXH motif, having two zinc-binding histidine residues and a glutamate residue that, along with the bound zinc ion, activates a water molecule that attacks a peptide bond of the protein being cleaved. Four members of this protease family (GtgA, GogA, and PipA from Salmonella enterica (2) and NleC from Escherichia coli (3)) have been shown to cleave proteins of the NF-κB complex to thwart the host immune response.

The NF-κB complexes consist of homo- and heterodimer assemblies of five proteins, p65, RelB, cRel, NF-κB1 (p105/p50), and NF-κB2 (p100/p52), whose distinct compositions differentially activate or inhibit transcription of different genes. All five subunits contain an N-terminal Rel homology region, which comprises N-terminal (NTD) and C-terminal dimerization (DD) immunoglobulin-like domains (4). Each dimer subunit binds in the DNA major groove.

Previous studies have reported that GtgA, GogA, and PipA can cleave the NF-κB subunits p65 and RelB, but cannot hydrolyze the p105/p50 and p100/p52 subunits (2), whereas NleC, whose sequence is less than 20% identical with those of the other three effectors, can cleave p65, RelB, cRel, and p105/p50 (3, 5). However, the mechanisms underlying the specificities of these four bacterial effectors for the different NF-κB subunits are unclear. Jennings et al. (6) now show that GtgA, GogA, and PipA also cleave cRel and that NleC can in fact cleave all five NF-κB subunits. Using X-ray crystallography and site-directed mutagenesis, the authors also identified the molecular recognition mechanism responsible for the differences in substrate affinity, expanding our understanding of how these effectors get a hold of and inactivate their specific target proteins.

Previous work has shown that although all four effector proteins cleave p65, they do so at different sites. GtgA, GogA, and PipA cleave p65 between residues Gly-40 and Arg-41 (2), whereas NleC cuts between residues Cys-38 and Glu-39 (3) (Fig. 1). Therefore, Arg-41 and Glu-39 can both be the P1’ residues (those located at the C-terminal end of the cleavage site), depending on which effector is cleaving p65. Using site-directed mutagenesis of FLAG-tagged p65, p50, and p100 expressed in GFP-, GtgA-, GogA-, or PipA-expressing human cells, Jennings et al. (6) found that this change in the P1’ residue was the cause of the differential substrate specificity. The reason for this is that Arg-41 of p65 is one of three residues surrounding the p65 cleavage sites that are not conserved in all NF-κB subunits. It is conserved in RelB and cRel, the other subunits for GtgA, GogA, and PipA, but the corresponding residue in the subunits not cleaved by these three effectors, p105/p50 and p100/p52, is a proline. In contrast, the P1’ residue Glu-39 in p65 recognized by NleC is conserved in all five NF-κB subunits, consistent with NleC being able to cleave all subunits.

Jennings et al. (6) solved the crystal structure of a catalytically inactive mutant of GtgA, with and without bound p65 NTD. They observed that the P1’ Arg-41 of p65 inserts into a negatively charged pocket of GtgA, explaining the specific preference of this effector for arginine over the proline present in the p105/p50 and p100/p52 subunits, which GtgA cannot cleave. A comparison with a previously reported NleC crystal structure (7) revealed that Arg-239 of NleC points toward the zinc ion in the active site and occupies a position equivalent to the S1’ of GtgA, consistent with NleC substrates having a glutamate at the P1’ position. It would be interesting to obtain a structure of an NleC–substrate complex to test whether Arg-239 is indeed part of the S1’ pocket, and to elucidate other interactions important for binding the substrates’ P1’ glutamate residue.

The GtgA–p65 complex structure indicated GtgA residues involved in interactions with p65, and single substitutions of these residues revealed that three of the four residues having an effect on the GtgA–p65 interaction are located in the S1’ pocket of GtgA and interact with Arg-41 of p65. It highlights

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2 The abbreviations used are: T3SS, type III secretion systems; RHR, Rel homology region; NTD, N-terminal domain; DD, dimerization domain.
that this pocket is important for providing both substrate specificity and affinity. Arg-221 substitution had the largest effect on GtgA’s catalytic activity, possibly by altering the position of the nearby Tyr-224 that helps stabilize the reaction intermediate.

Finally, the crystal structures presented by Jennings and colleagues (6) and the previously reported NleC structure confirmed previous suggestions that these effector proteins mimic the major groove of DNA, which is the normal binding target for the NF-κB subunits (8). Specifically, the shapes of the active-site clefts of GtgA and NleC are similar to that of the major groove and are lined with acidic charges as in DNA. Moreover, Jennings and co-workers’ GtgA–p65 complex structure disclosed that the C-terminal region of the NTD of p65 is positioned such that the second immunoglobulin-like DD of p65 can be bound by a second major groove-like cleft on the GtgA surface, consistent with how p65 binds to DNA.

As other homologs of these effector proteins are discovered, it will be interesting to see how much variation in NF-κB subunit substrate specificity they may have. Such differences would likely produce changes in the transcription of different subsets of genes in the host. For instance, it is known that p50 and p52 homodimers have defined gene-repressing and -activating effects. Therefore, NleC, being able to cleave these subunits, would alter these effects, but GtgA, GogA, and PipA would not.

We anticipate that besides significantly advancing the field of microbe–host interactions, the detailed molecular level insights reported by Jennings and colleagues (6) will have implications for the development of strategies targeting bacterial effectors that interfere with host immune proteins.

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Figure 1. Cleavage of NF-κB subunits by bacterial T3SS zinc metalloprotease effector proteins. GtgA and NleC (represented in the cartoon with the zinc ion as a green sphere, water as a red sphere, and zinc binding and catalytic glutamate residues as sticks) have been shown to cleave p65 (depicted as surface structures with the NTD in green and the DD in cyan) at the peptide bond before Arg-41 (blue) and Glu-39 (red), respectively. This cleavage releases a short peptide (light purple) from the p65 N terminus, which previously threaded through the center of the p65 structure, and thereby disrupts it, preventing DNA binding.