Thinking outside the Osp(G)—kinase activation by E2-ubiquitin

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OspG is a secreted effector kinase from the human pathogen *Shigella* that is required for the reduction of immune responses during *Shigella* infection. A new study in *The EMBO Journal* provides a co-crystal structure of OspG bound to Ubch5c~Ub, revealing how a bacterial kinase can be activated by the host ubiquitin conjugation machinery. These results provide molecular insight into an enigmatic microbial virulence factor that thwarts the host immune surveillance system to cause disease.

See also: J Pruneda *et al* (March 2014)

One of the hallmarks of bacillary dysentery (or shigellosis) is invasion of the colonic and rectal mucosa by *Shigella* spp., a Gram-negative gamma-proteobacterium that induces severe inflammation, mucosal destruction, abscess formation and mucosal hemorrhages in infected humans (Speelman *et al*, 1984; Raqib *et al*, 1995). Despite the fact that inflammation is a hallmark of this disease, recent studies have suggested that *Shigella* encodes virulence factors that actually dampen cellular signal transduction pathways that produce pro-inflammatory cytokines. However, very little is known about the molecular interplay between the host and pathogen molecules involved in these processes. In an elegant series of biochemical, structural, and *in vivo* studies presented in *The EMBO Journal*, Pruneda *et al* (2014) demonstrate how the *Shigella* type III secreted effector protein OspG couples the host ubiquitin conjugation system to bacterial kinase activation, and subsequent inhibition of the host inflammatory response (Fig 1). In doing

so, this work establishes a novel signaling circuit required for immune evasion by an important class of human pathogens.

Prior to their work, OspG was shown to be encoded by the virulence plasmid of all *Shigella* serogroups and was found to be a type III secreted effector protein (T3SS) (Buchrieser *et al*, 2000). OspG has sequence similarity to eukaryotic protein kinases of the RIO family, however it lacks several kinase domain modules including the activation loop. Although the kinase target of OspG remains unknown, OspG was previously shown to interact with Ubch5b~Ub, a host ubiquitin-conjugating (E2) enzyme carrying activated ubiquitin (Ub) (Kim *et al*, 2005), and this interaction was shown to increase kinase activity (Kim *et al*, 2005; Zhou *et al*, 2013). However, the molecular mechanism of this stimulatory process was previously unknown. In addition, one observed phenotype for a *Shigella* ospG mutant during infection of tissue culture cells or in the rabbit model of infection is the increase in inflammation compared to infection with wild-type *Shigella* (Kim *et al*, 2005). This phenotype has been linked to the ability of OspG to reduce degradation of I κ B α , which functions as an inhibitor in the NF- κ B pathway (Kim *et al*, 2005; Zhou *et al*, 2013). However, no direct link has been demonstrated yet between OspG functioning to reduce inflammation during infection and OspG kinase activity or binding to E2~Ub.

The work by Pruneda *et al* delivers significant insight into the interaction between *S. flexneri* OspG and E2~Ub conjugates and the implication of this interaction for OspG kinase function beyond previous descriptions (Kim *et al*, 2005; Zhou *et al*, 2013). Importantly, the crystal structure of the

OspG/UbcH5c~Ub complex was determined to a resolution of 2.70 Å, which has provided near-atomic resolution view of this unusual bacterial kinase and its regulation by the host ubiquitin system. Like most kinases, OspG contains an N-terminal and a C-terminal lobe connected through a hinge. Unexpectedly, both Ub and UbcH5c bind to OspG at different surfaces. UbcH5c contacts mostly residues in the N-terminal lobe and Ub seems to bind only to the C-terminal lobe. In comparison to structurally related mammalian kinases there are few contacts between the two lobes, however the interaction with Ubch5c~Ub appears to stabilize the OspG N-terminal and C-terminal lobes and the active site. This observation from the co-crystal structure is supported by the finding from *in vitro* experiments that OspG only weakly phosphorylates the surrogate substrate histone when alone, but shows increased activity when co-incubated with E2~Ub. In addition, mutation of a cysteine residue to an arginine at the binding interface with Ub reduced kinase activity of OspG when present together with UbcH5c~Ub.

The formation of an OspG/UbcH5c~Ub complex not only stabilizes OspG in the active conformation, but also seems to reduce the ability of Ub to be transferred to lysine residues in the presence of E3 ligating enzymes. Indeed, the OspG co-crystal structure shows the UbcH5c~Ub conjugate is held in an extended conformation compared to free UbcH5c~Ub, and it was further shown that the E2~Ub conjugate was more stable in the presence of OspG. These data suggest that the E2~Ub conjugate bound to OspG is less likely to transfer its ubiquitin to a substrate in the presence of a RING E3. This suggests that OspG forms a stable complex

Figure 1. Schematic representation of OspG secretion into host cells through the *Shigella* Type III secretion system.

In the host cell, interaction of both N-terminal and C-terminal lobes of OspG with E2~Ub conjugates results in an active kinase conformation (see inset), and mediates OspG kinase activation and down-regulation of the inflammatory response, likely involving phosphorylation of yet to be identified substrate(s). The structural model shown in the inset is reproduced from the original article by Pruneda *et al* (2014) with the authors' permission.

with E2~Ub conjugate to (1) activate and prolong its kinase activity and (2) prevent competition with natural ubiquitination reactions.

While the authors' structural work was focused on the OspG/UbcH5c~Ub complex, they also demonstrated that OspG binds to a panel of different E2~Ub conjugates. Closer inspection of the OspG-UbcH5c interaction surface in the co-crystal structure revealed that all interacting E2s have similar residues contacting OspG. OspG was also shown to be able to bind to unconjugated Ub (Zhou *et al*, 2013), however this interaction or the interaction of OspG with unconjugated E2 appears to be much weaker. In addition kinase activity of OspG was not enhanced as much in *in vitro* assays when incubated with unconjugated Ub or E2 compared to E2~Ub conjugates. Thus OspG could potentially bind to many different E2~Ub conjugates during *Shigella* infection. While it is currently unclear if this broad specificity has biological

relevance, one could speculate that the ability of multiple E2~Ub conjugates to activate OspG would increase the robustness of this *Shigella* immune evasion strategy.

To get more insight into the OspG interaction with E2~Ub during *Shigella* infection, the authors utilized a murine model of shigellosis. Mice were orally infected with five different *S. flexneri* strains including wild-type, $\Delta ospG$ or $\Delta ospG$ expressing a wild-type copy ($ospG^{wt}$), a copy containing mutations of residues at the E2~Ub binding interface ($ospG^{mut}$) or a copy that has been mutated to be catalytically deficient ($ospG^{cd}$). Although the conclusions from these experiments will require further validation, they found, intriguingly, that colons from wild-type mice showed less inflammation than those infected with $\Delta ospG$ or the $ospG^{mut}$ strain. These data suggest that the ubiquitin conjugation system in mammals is required for OspG function during infection. However, the link between OspG

binding E2~Ub and kinase activation *in vivo* was less clear. Nevertheless, these data provide compelling evidence for a concerted regulatory circuit, dependent on the host ubiquitin conjugation machinery, which enhances the OspG bacterial type III effector protein activity *in vivo*.

In summary, the work by Pruneda *et al* provides important molecular insights into how catalytic activity of the minimal kinase OspG from *Shigella* is enhanced upon binding a host E2~Ub conjugate. Although several questions still remain (including the identity of the ubiquitin-dependent kinase substrate), it is now clear that the precise timing and localization of OspG function is important for *Shigella* infection.

Conflict of interest

The authors declare that they have no conflict of interest.

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