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Lost after translation: post-translational modifications by bacterial type III effectors

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Abstract

Many Gram-negative bacterial pathogens use the type III secretion system to deliver effector proteins into host cells. These effectors use various mechanisms to exploit host processes to the advantage of the pathogen. A large group of effectors use post-translational modifications, either reversible or irreversible, to manipulate host proteins, and while most of these mechanisms mimic eukaryotic activities, others appear to be unique biochemical functions. Deciphering such mechanisms and identifying the host targets of these effectors sheds light on eukaryotic signaling pathways and immune responses.

Introduction

Bacterial pathogens are a major cause of disease in animals and plants. A major virulence determinant found in many Gram-negative bacteria is a secretory apparatus called the type III secretion system (T3SS) [1]. The T3SS is used to translocate a suite of effector proteins into the cytoplasm of host cells, where they manipulate host cellular functions to the advantage of the pathogen [2,3]. While the repertoire of bacterial effectors varies between bacterial strains, they use similar mechanisms to target eukaryotic signaling pathways, often by mimicking eukaryotic biochemical processes. Some of the mechanisms used by bacterial effectors are post-translational modifications that target host proteins to activate or inhibit their activity. Such modifications can be either reversible or irreversible. Many effectors manipulate common targets that are required for host immune responses, such as mitogen activated protein kinase (MAPK) pathways, Rho and Ras GTPase signaling cascades, the ubiquitin-proteasome system, and the cytoskeleton. Other effectors manipulate host targets to gain access into the cell and maintain a niche in which they can thrive. Elucidating how bacterial effectors modify host signaling and which host pathways are targeted is necessary to understand bacterial pathogenesis and can also be used as a tool to study eukaryotic processes.

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Ethics statement

The authors declare that no competing interests exist.

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Modifications using small molecule substrates

Phosphorylation and de-phosphorylation

The first described bacterial effector with a kinase activity was the *Yersinia* YpkA/YopO (Figure 1a) [4]. YpkA/YopO has homology to eukaryotic serine/threonine kinases and contains a domain that mimics host guanidine nucleotide dissociation inhibitors (GDIs) [4,5]. The kinase activity of YpkA/YopO inhibits Gαq signaling pathways in host cells by phosphorylating a serine residue in the conserved diphosphate-binding loop of the active GTP-bound form of Gαq [6]. This phosphorylation decreases the affinity of Gαq for GTP, possibly due to a conformational change, thus preventing G-protein activation (Figure 1a) [6]. In addition, the YpkA/YopO kinase activity is positively regulated by binding host monomeric (G) actin, thereby, preventing promiscuous activity while residing in *Yersinia* [7,8].

Another bacterial effector kinase is the *Shigella* OspG that exhibits similarities to eukaryotic kinase domains but lacks subdomains VIII–XI [9]. While a target of OspG phosphorylation remains unknown, its kinase activity is required to prevent phospho-IκBα degradation and NFκB activation induced by TNF-α stimulation, resulting in interference of host innate immune responses [9].

As the phosphorylation state of a protein can regulate its activity, it is not surprising that bacterial effectors can not only phosphorylate host proteins, but also de-phosphorylate them to manipulate their function. The *Yersinia* bacterial effector YopH is a highly active protein-tyrosine phosphatase that is essential for virulence by targeting various signaling pathways required for host immunity [10]. YopH inhibits uptake of *Yersinia* by host cells through disruption of peripheral focal adhesion complexes [11] and suppresses T and B lymphocyte activation [12]. Several substrates of YopH have been detected and include phosphorylated tyrosine kinases and adaptors, such as p130Cas, FAK, Fyb, and p85 [11,13,14].

Phytopathogenic bacteria also use phosphatases. The *Pseudomonas syringae* bacterial effector HopAO1 (HopPtoD2) is a protein-tyrosine phosphatase [15,16] that suppresses innate immunity in *Arabidopsis*, presumably by targeting a step downstream or independent of mitogen activated protein kinase (MAPK) activation [17].

Acetylation

The *Yersinia* bacterial effector YopJ inhibits the host inflammatory response and promotes apoptosis of immune cells by blocking multiple signaling pathways, including the MAPK and NFκB pathways (Figure 1b) [18]. While YopJ was initially believed to be a protease based on its catalytic domain being similar to that of Clan CE of cysteine proteases [19], it was found to be an acetyltransferase that uses acetyl-coenzyme A (CoA) to modify serine and threonine residues on the activation loop of MAPK kinases (MAPKKs) and NFκB pathway kinase, IKK (Figure 1b) [20,21]. Although the chemistry for a protease and a transferase are the same, the substrates used in each reaction are different [22]. This modification blocks phosphorylation and thus activation of the target protein kinases responsible for cytokine production and other immune responses [21,23].

Several other members of the YopJ-superfamily are also acetyltransferases. For example, the *Vibrio* effector VopA potently inhibits MAPK signaling pathways by acetylating not only the conserved serine and threonine on the activation loop of MAPKKs, but also a conserved lysine found in the catalytic loop of MAPKKs [24]. These modifications prevent both the activation of MAPKKs, as well as the activity of activated MAPKKs [24]. However, in contrast to YopJ that promotes apoptosis via the inhibition of the NF κ B pathway by the primarily extracellular *Yersinia* spp., VopA does not inhibit the NF κ B pathway, thus avoiding induction of apoptosis. Notably, since VopA is expressed by the invasive *Vibrio parahaemolyticus*, promoting apoptosis in the host cell in which it is residing and proliferating is unfavorable [25].

Recently, the *Pseudomonas syringae* bacterial effector HopZ1a was shown to be an acetyltransferase that is activated by the co-factor phytic acid [26]. Upon activation, HopZ1a acetylates itself and tubulin in plant cells causing a dramatic decrease in microtubule networks, disruption of the plant secretory pathway and suppression of cell wall-mediated defenses [26].

ADP-ribosylation

The *Pseudomonas aeruginosa* bacterial effector ExoS is an ADP-ribosyltransferase that is homologous to vertebrate mono-ADP-ribosyltransferases [27]. ExoS ADP-ribosylates Ras on more than one arginine residue, resulting in inhibition of Ras-mediated signal transduction [28]. Another *Pseudomonas aeruginosa* bacterial effector, ExoT, ADP-ribosylates Crk-I and Crk-II, which are proteins that mediate signal pathways involving focal adhesion and phagocytosis (Figure 1c) [29]. The modification of the Crk adaptor molecules by ExoT is speculated to disrupt the assembly of the focal adhesion complex resulting in the anti-phagocytosis phenotype in infected mammalian cells [29]. VopT, a bacterial effector from *Vibrio parahaemolyticus*, is also an ADP-ribosyltransferase that targets Ras and shares approximately 45% and 44% identity with the ADP-ribosyltransferase domain of ExoT and ExoS, respectively [30]. However, the effect of VopT-mediated ADP-ribosylation on pathogenicity remains unknown.

Plant pathogens also use ADP-ribosyltransferases to manipulate host signaling. HopU1 from *Pseudomonas syringae* is a mono-ADP-ribosyltransferase that suppresses plant innate immunity [31]. HopU1 targets RNA-binding proteins that possess RNA-recognition motifs, possibly to interfere with their ability to bind RNA and thus affect metabolism of plant defense related RNA [31]. Another *Pseudomonas syringae* ADP-ribosyltransferase, the bacterial effector HopF2, targets *Arabidopsis* MAPKKs thereby inhibiting the ability of the infected plant cells to elicit innate immunity responses [32].

AMPylation and UMPylation

A novel post-translational modification was recently “rediscovered” in the *Vibrio* effector VopS (Figure 1d) [33]. This effector contains a domain called filamentation induced by cyclic AMP (Fic) that is found in a variety of species including eukaryotes [33,34]. The Fic domain was found to covalently modify a conserved threonine residue on the family of Rho GTPases, including Rho, Rac, and Cdc42 with adenosine 5'-monophosphate (AMP) [33].

This post-translational modification was named AMPylation to distinguish it from adenylation which is a mechanism used to charge an enzymatic reaction [35]. The inhibition of Rho GTPases by AMPylation results in cell rounding and collapse of the actin cytoskeleton (Figure 1d) [33]. In the early 1970s, Earl Stadtman and colleagues observed that a bacterial nucleotidyltransferase could reversibly modify a protein with AMP, however, the field remained dormant for almost 40yrs [36]. Since the discovery of VopS, the field of study whereby a target protein is modified by AMP mediated by either a Fic domain or a nucleotidyltransferase has exploded [35]. Like kinases, Fic domains or nucleotidyltransferases can also modify tyrosine residues on target proteins, as shown for the Ficdomain found in the secreted toxin IbpA from *Histophilus somni* [37]. Interestingly, a bioinformatic analysis using sequence and structure-based similarity detection methods revealed a similar topology for fic, doc and AvrB domains, now referred to as Fido [34]. Interestingly, Fic domains can use substrates other than AMP to modify target proteins. The Fic domain containing effector AvrAC from the phytopathogen *Xanthomonas campestris* uses UTP as a substrate, resulting in UMPylation of two *Arabidopsis* receptor-like cytoplasmic kinases, BIK1 and RIPK [38]. This modification competes with phosphorylation on the activation loops by upstream kinases to enhance virulence and inhibit plant immunity [38]. This is similar to the competitive post-translational modification strategy used by the acetyltransferase YopJ to inhibit host-mediated phosphorylation of kinases.

Small-protein modifications

Ubiquitination and de-ubiquitination

The host ubiquitin-proteasome system (UPS) plays a major regulatory role in many eukaryotic cellular processes and is therefore a lucrative target for bacterial virulence factors [39]. For example, the bacterial effector AvrPtoB from *Pseudomonas syringae* functions as an E3 ubiquitin ligase (Figure 2) [40,41]. While its amino acid sequence provides no clues as to its possible function, AvrPtoB is structurally homologous to RING-finger E3 ubiquitin ligases [40,41]. AvrPtoB uses this E3 ubiquitin ligase activity to target plant pattern-recognition receptors for degradation, resulting in suppression of plant cell death and immunity (Figure 2) [42,43]. Similarly, the *Salmonella* effector SopA and the *E. coli* NleL mimic the mammalian HECT family of E3 ubiquitin ligases, both of which have been shown to contribute to pathogenicity, although their host targets have not been elucidated [44,45].

Effectors in the IpaH family also have an E3 ubiquitin-ligase activity [46], however their structure differs from the cononical RING- and HECT domains [47]. IpaH9.8 expressed by *Shigella* hampers NF κ B-mediated inflammatory response to bacterial infection by promoting ABIN-1-dependent polyubiquitynation of an upstream signaling component in the NF κ B pathway, NEMO/IKK γ , resulting in degradation of this scaffold by the proteasome [48]. Similar to what is observed with ExoT, a scaffold, rather than the enzyme, is targeted, resulting in an inactive signaling complex.

Another way to manipulate the UPS is to de-ubiquitinate a target host protein. The *Salmonella* effector SseL is a deubiquitinase [49] that prevents accumulation of cellular lipid droplets, suggesting that it alters host lipid metabolism by modifying the ubiquitination

patterns of cellular targets [50]. Furthermore, SseL inhibits the accumulation of ubiquitinated protein aggregates associated with the *Salmonella*-containing vacuole and deubiquitinates substrates targeted for autophagic degradation [51].

De-SUMOylation

Ubiquitination is only one of many small-protein modifications that regulate protein activity in eukaryotic cells. Another example of which is SUMOylation, where SUMO (Small Ubiquitin-like Modifier) is added via an isopeptide-bond to a target protein [52]. The *Xanthomonas campestris* XopD is an example of a bacterial effector that encodes multiple domains, including an active cysteine protease with specificity for proteins modified with plant SUMO [53,54]. XopD binds the *Arabidopsis* transcription factor MYB30, which is a substrate for SUMOylation *in vitro* [55], to suppress plant defenses [56]. However, the catalytic activity of XopD is not required for this suppressive effect [56]. Therefore, further investigation on how this multi-domain effector is utilized by *Xanthomonas* is required.

Irreversible cleavage and proteolysis

Proteases

A common mechanism for irreversible post-translational modification utilized by bacterial effectors to inhibit protein activity is hydrolysis of host proteins. The *Yersinia* YopT is a member of a cysteine protease family found in bacteria that infect animals and plants [57]. YopT requires an invariant C/H/D catalytic triad to cleave lipid-modified Rho GTPases near their carboxyl termini [57]. This cleavage causes Rho GTPases to be released from the membrane, thereby disrupting the membrane-associated signal transduction that regulates the actin cytoskeleton [57]. AvrPphB, a YopT family member from the plant pathogen *Pseudomonas syringae*, is also a cysteine protease [57]. AvrPphB cleaves itself inside the plant cell, exposing a myristoylation motif. After myristoylation, AvrPphB localizes to the plasma membrane [58] where it cleaves its host substrate, the membrane-bound plant serine/threonine kinase PBS1 [59]. Interestingly, plants have evolved “guard” proteins that can detect bacterial effector-mediated proteolysis of host targets resulting in the activation of immune responses [60]. Such is the case for the cleavage of PBS1 by AvrPphB where the cleaved PBS1 is recognized by RPS5, a plant resistance protein that subsequently activates the plant immune response (Figure 3a) [59].

The two *Escherichia coli* effectors NleC and NleD are Zn-dependent proteases that share the conserved Zn metalloprotease signature motif, HEXXH [61]. NleC cleaves RelA (p65) while NleD cleaves the c-Jun N-terminal kinase (JNK) thus blocking both the NF κ B and JNK pathways, resulting in inhibition of the host inflammatory response [61].

The *Chlamydia* effector CPAF, an autoprocessing and self-activating serine protease [62], has broad substrate specificity and attacks a wide spectrum of host proteins [63]. A recent study demonstrated that CPAF also targets chlamydial effectors that are secreted early during the establishment of the pathogen-containing vacuole [64]. It is possible that the cleavage of these chlamydial effectors in the later stages of infection is essential for the intracellular chlamydial life-cycle [64].

Phosphothreonine-lyases

The *Shigella* effector OspF and the *Salmonella* SpvC inactivate MAPKs using a unique mechanism (Figure 3b). These bacterial effectors are phosphothreonine lyases that carry out a β -elimination reaction to irreversibly remove the phosphate moiety from the phosphothreonine in the pT-X-pY motif of phosphorylated MAPKs. This modification results in an irreversibly inactive kinase [65,66] because the modified MAPKs can no longer be activated by phosphorylation due to the loss of the hydroxyl moiety on the threonine residue [65]. Another member of the phosphothreonine lyase family, HopAI1, is found in the plant pathogen *Pseudomonas syringae*. HopAI1 inactivates plant MAPKs using the same mechanism, resulting in suppression of the plant innate immunity [65,67].

De-amidation

VopC is a bacterial effector found in *Vibrio* species, that induces the formation of actin ruffles and filopodia in host cells to mediate bacterial invasion (Figure 3c) [25]. This effector contains a catalytic domain that is similar to the one found in cytotoxic necrotizing factor toxins. In VopC, a short type III secretion signal replaces the large N-terminal part of the toxins, which is responsible for the toxins secretion, membrane binding, and internalization into the target cell. VopC de-amidates glutamine 61 in the small G proteins Rac and Cdc42, resulting in their constitutive activation [25], similar to the mechanism reported for the *E. coli* cytotoxic necrotizing factor 1 [68].

Conclusions

Bacterial effectors use various mechanisms to manipulate their eukaryotic host to their advantage. Many of these mechanisms are post-translational modifications of host proteins, and occasionally of co-secreted bacterial proteins. Deciphering these mechanisms and identifying the host targets of these bacterial effectors has helped to shed light on eukaryotic signaling pathways and widened our understanding of host immune responses and cellular processes. It is relevant to point out that many effectors contain complex, overlapping information to mediate efficient virulence, including but not limited to secretion signals, localization motifs, substrate recognition domains, catalytic domains and binding domains.

In recent years, the advent of mass-spectrometry has revolutionized the study of effector-mediated post-translational modifications. This technology enables scientists to confirm and discover small molecular weight modifications imposed on target host proteins. Several examples for this type of discovery are discussed in this review including YopJ, VopS, OspF, and VopC.

It is interesting to note that while some of the effectors modify their targets by reversible mechanisms (*e.g.* phosphorylation), others use an irreversible mechanism (*e.g.* proteases) to modify their targets. It is compelling to speculate that a bacterium will “choose” an effector with a reversible or irreversible mechanism of modification based on the nature of the target protein and the manner in which the pathogen needs to manipulate the host. If the target requires constant activation or inhibition, the bacterium would gain from using an effector that modifies it irreversibly. For example, the phytopathogen *Pseudomonas syringae* uses the

phosphothreonine lyase HopAI1 to irreversibly modify and inhibit MAPKs. Since activation of many plant defense responses are mediated by MAPK pathways, the pathogen must constantly inhibit host MAPK cascades. Using an irreversible inhibition mechanism is a far more efficient mechanism to maintain constant inhibition. On the other hand, some pathogens may require only temporary manipulation of a host protein to assist them in creating a niche, but would benefit from normal activity of that protein in later stages of the infection. In this case, the pathogen would preferentially use an effector that modifies its target in a reversible manner so that the activity could be restored. Examples for this type of manipulation would include phosphorylation and possibly AMPylation.

Importantly, the potent and sometimes unique and irreversible nature of bacterial effector-mediated post-translational modifications can also be used to compile a synthetic tool box that will assist us in controlling eukaryotic cellular processes. An exciting proof of concept was recently presented by Wei and colleagues, who used the bacterial effectors OspF and YopH (discussed in this review), to rewire and fine tune kinase pathways in T-cells [69]. As new technologies and pathogens arise, we can expect more advances and discoveries in the field of microbial pathogenesis.

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Highlights

- Type III effectors manipulate host proteins using post-translational modifications.
- Effectors can activate or inhibit the activity of target proteins.
- The study of type III effectors shed light on eukaryotic signaling pathways.
- Type III effectors can be used to compile a synthetic tool box.

Figure 1.

Post-translational modifications using small molecule substrates. **(a)** YpkA phosphorylates an active GTP-bound form of Gαq to prevent G-protein activation. **(b)** MAPKK and IKK are activated upon phosphorylation of serine and threonine residues in the activation loop by MAPKKKs. YopJ acetylates these residues, thereby preventing their phosphorylation and activation. **(c)** ExoT ADP-ribosylates CrkI and CrkII. This modification disrupts the assembly of the focal adhesion complex and phagocytosis. **(d)** VopS uses ATP to AMPylate Rho-family GTPases and inhibit their activity, resulting in collapse of the actin cytoskeleton.

Figure 2. Small protein post-translational modifications. AvrPtoB poly-ubiquitinates pattern recognition receptors (PRRs) resulting in their degradation by the proteasome. Eliminating PRRs hampers the cell's ability to recognize invading bacteria and activate immune responses.

Figure 3.

Irreversible post-translational modifications. **(a)** AvrPphB cleaves the plant kinase PBS1. RPS5 functions as a “guard” that recognizes the cleaved PBS1 and elicits an immune response. **(b)** OspF carries out a β -elimination reaction that irreversibly removes the phosphate from phosphorylated MAPKs, resulting in an inactive kinase that can no longer be activated. **(c)** VopC de-amidates a glutamine residue on Rho-GTPases to activate them and mediate bacterial invasion.

Table 1

Effectors discussed in this paper and their enzymatic activities

Effector	Activity	Bacterium
YpkA/YopO	Kinase	<i>Yersinia spp.</i>
OspG	Kinase	<i>Shigella spp.</i>
YopH	Phosphatase	<i>Yersinia spp.</i>
HopAO1	Phosphatase	<i>Pseudomonas syringae</i>
YopJ	Acetyltransferase	<i>Yersinia spp.</i>
VopA	Acetyltransferase	<i>Vibrio parahaemolyticus</i>
HopZ1a	Acetyltransferase	<i>Pseudomonas syringae</i>
ExoS	ADP-ribosyltransferase	<i>Pseudomonas aeruginosa</i>
ExoT	ADP-ribosyltransferase	<i>Pseudomonas aeruginosa</i>
VopT	ADP-ribosyltransferase	<i>Vibrio parahaemolyticus</i>
HopU1	ADP-ribosyltransferase	<i>Pseudomonas syringae</i>
HopF2	ADP-ribosyltransferase	<i>Pseudomonas syringae</i>
VopS	AMPylation	<i>Vibrio parahaemolyticus</i>
AvrAC	UMPylation	<i>Xanthomonascampestris</i>
AvrPtoB	E3 ubiquitin ligase	<i>Pseudomonas syringae</i>
SopA	E3 ubiquitin ligase	<i>Salmonella spp.</i>
NleL	E3 ubiquitin ligase	<i>Escherichia coli</i>
IpaH	E3 ubiquitin ligase	<i>Shigella spp.</i>
SseL	De-ubiquitinase	<i>Salmonella spp.</i>
XopD	De-SUMOylation	<i>Xanthomonascampestris</i>
YopT	Protease	<i>Yersinia spp.</i>
AvrPphB	Protease	<i>Pseudomonas syringae</i>
NleC	Protease	<i>Escherichia coli</i>
NleD	Protease	<i>Escherichia coli</i>
CPAF	Protease	<i>Chlamydia spp.</i>
OspF	Phosphothreoninylase	<i>Shigella spp.</i>
SpvC	Phosphothreoninylase	<i>Salmonella spp.</i>
HopAI1	Phosphothreoninylase	<i>Pseudomonas syringae</i>
VopC	De-amidase	<i>Vibrio parahaemolyticus</i>