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Post-transcriptional regulation of type III secretion in plant and animal pathogens

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Abstract

Type III secretion systems (T3SS) serve as a primary anti-host defense mechanism for many Gram-negative plant and animal pathogens. T3SS production is tightly controlled and activated by host-associated signals. Although transcriptional responses represent a significant component of the activation cascade, recent studies have uncovered diverse post-transcriptional mechanisms that also contribute to T3SS production. Targets for post-transcriptional control are often AraC/XylS transcription factors that promote T3SS gene expression. Common mechanisms of post-transcriptional regulation include direct control of either the activity of AraC/XylS transcription factors by protein ligands, small molecules, or post-translational modification, or transcription factor synthesis. In the latter case, RNA-binding proteins such as Hfq, CsrA/RsmA, and components of the RNA degradosome alter mRNA stability and/or the rate of translation initiation to control transcription factor synthesis. Here we summarize post-transcriptional mechanisms that contribute to the exquisite regulation of T3SS gene expression.

Keywords

type III secretion; post-transcriptional; Hfq; CsrA/RsmA; sRNA; RNA helicase

INTRODUCTION

Most bacterial pathogens are versatile microorganisms found in water, soil, host, and/or other environments. Survival under these ever-changing conditions necessitates rapid adaptive responses to nutrient availability, stressors, and host defense mechanisms. Type III secretion systems constitute an important anti-host response in many Gram-negative pathogens. The T3SS machinery consists of a conserved core structure spanning the cell envelope that functions to assemble a needle-like injectisome complex [1]. The injectisome promotes virulence by translocating effector proteins into host cells where they exert anti-host effects such as facilitated invasion, intracellular survival, phagocytic avoidance, and disruption of host defenses [2]. T3SS production is tightly controlled at the transcriptional

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and post-transcriptional levels in response to a variety of environmental cues [3]. Although transcription of T3SS genes has been studied extensively, there is growing appreciation for regulatory events occurring at the post-transcriptional level. Common, but not exclusive, targets for post-transcriptional control are the AraC/XylS transcription factors that regulate T3SS gene transcription and the secreted T3SS effectors. Mechanisms of post-transcriptional control include regulation of transcription factor activity, and transcription factor or effector protein synthesis.

Post-transcriptional Control of AraC/XylS Transcription Factor Activity

T3SSs consist of >25 genes encoding structural components of the secretion apparatus, translocators, effectors, and transcription factors [4]. A common feature of T3SS gene regulation is involvement of one or more AraC/XylS transcription factors [5]. Depending upon the microorganism the AraC/XylS factors control either a subset of the T3SS genes or all T3SS-associated functions [6]. AraC/XylS proteins typically consist of an amino-terminal domain that can serve as an input for regulatory control and a carboxy-terminal DNA-binding domain [7]. Modulating the activity of AraC/XylS transcription factors by post-transcriptional events is important in the control of T3SS gene expression. Mechanisms include partner-switching, interactions with small molecules, and tyrosine phosphorylation.

Partner-switching mechanisms controlling transcription factor activity—T3SSs have been described as contact-dependent secretion systems because intimate contact between the pathogen and the host cell triggers secretory activity and subsequent T3SS gene transcription [8]. In *Shigella flexneri*, *Pseudomonas aeruginosa*, and several other pathogens T3SS gene expression is coupled to secretion by partner-switching mechanisms [9,10]. The central player in partner-switching systems is a dual-function protein that serves as both a chaperone for a secreted protein and a positive-acting factor for T3SS transcription [11]. In the absence of host cell contact, the secretion substrate and chaperone form a complex that inhibits the positive-acting function of the chaperone [12]. Host cell contact triggers secretion/translocation activity and release of the chaperone from the secretion substrate allowing the chaperone to exert its positive effect on T3SS gene expression [13].

The *P. aeruginosa* partner-switching system consists of four proteins. ExsA, an AraC/XylS protein, is the primary activator of *P. aeruginosa* T3SS gene expression (Fig. 1A) [14]. The DNA-binding activity of ExsA is antagonized through a direct interaction with the anti-activator ExsD [15]. The dual function protein ExsC serves as a chaperone for the secreted substrate ExsE and as an anti-anti-activator [11,16]. Prior to host cell contact, ExsA-ExsD and ExsC-ExsE are preferentially bound to one another (Fig. 1Ai). Activation of ExsE secretion by host cell contact releases ExsC and triggers partner-switching wherein ExsC directly interacts with and sequesters ExsD thereby releasing ExsA to activate T3SS expression (Fig. 1Aii) [17].

The *S. flexneri* partner-switching system is more complex and involves two dual-function chaperones, IpgC and Spa15 (Fig. 1B). IpgC is a chaperone for the IpaB/C translocator proteins and a co-activator of MixE, an AraC/XylS protein that activates effector gene transcription (Fig. 1B) [18]. Spa15 functions as a chaperone for OspD1 and as a co-anti-

activator that together with OspD1 inhibits MxiE activity [18]. Secretion of IpaB/C and OspD1 liberates IpgC to serve as a co-activator for MxiE and also relieves the inhibitory block on MxiE imposed by Spa15/OspD1 (Fig. 1Bii) [18].

Inhibition of transcription factor activity by small molecular weight

compounds—Transcription of the T3SS located on *Salmonella enterica* serovar Typhimurium pathogenicity island I (SPI1) is controlled by several AraC/XylS transcription factors including HilD. HilD is a primary target of post-transcriptional control. The SPI1 T3SS genes are preferentially expressed in the small intestines and repressed by long-chain fatty acids [19]. A recent study found that the long chain fatty acid oleate inhibits HilD DNA-binding activity in vitro (Fig. 1C) [20]. It has been proposed that long-chain fatty acids are absorbed along the length of the intestines. When *S. enterica* reaches the distal ileum the concentration of long-chain fatty acids falls below the critical concentration required to inhibit HilD activity and this contributes to the activation of SPI1 gene expression [20].

Inhibition of transcription factor activity by tyrosine phosphorylation—

Although tyrosine phosphorylation is as an important post-transcriptional protein modification in eukaryotes, roles in prokaryotic cells are only starting to be revealed. A recent study in *S. flexneri* identified tyrosine phosphorylation events for almost 600 proteins including VirB and Spa47 [21]. VirB together with MxiE are the primary activators of *S. flexneri* T3SS transcription. Spa47 is an ATPase and a key structural component of the T3SS. Phosphorylation of VirB at tyrosine 1000 and Spa47 at tyrosine 247 impairs VirB-dependent expression of the T3SS and T3SS function, respectively (Fig. 1D). How the modifications inhibit Spa47 and VirB functions (i.e., protein stability or activity), and whether the phosphorylation events are regulated remain to be determined.

Stability of Transcription Factor mRNA

Role of the RNA-degradosome—Regulated mRNA degradation drives many adaptive responses. Polynucleotide phosphorylase (PNPase) is a 3' exoribonuclease that can function independently or as part of the larger RNA degradosome complex that consists of PNPase, RNase E, enolase, and a DEAD-box helicase [22]. The RNA degradosome/PNPase can exert either negative or positive effects on the T3SS. For instance, the 3' untranslated region of *S. enterica hilD* mRNA is unusually long (310 nt) and is targeted by the RNA degradosome resulting in reduced mRNA stability and T3SS gene expression (Fig. 2A) [23]. Similarly, PNPase negatively regulates T3SS in the phytopathogen *Dickeya dadantii* by reducing the half-life of the *hrpL* mRNA, which codes for an AraC/XylS activator of T3SS [24]. Whether these activities simply reflect housekeeping functions or bona fide regulatory events is unclear.

In addition to targeting T3SS transcription factors, the RNA degradosome can also target genes encoding T3SS structural components. In enterohemorrhagic *Escherichia coli* the genes encoding the T3SS translocon (*espADB*) are immediately preceded by a mini-ORF containing 6 codons [25]. Occupation of the mini-ORF Shine Dalgarno sequence by ribosomes protects the *espADB* mRNA from RNase E-mediated degradation while

mutations that reduce ribosome binding, but not mini-ORF translation, increase susceptibility to RNase E degradation (Fig. 2B).

Hfq and small non-coding RNAs—Hfq is an RNA chaperone that promotes base pairing between small non-coding regulatory sRNAs and mRNA targets [26]. Hfq can exert both negative and positive effects on T3SS gene expression though few direct mechanisms have been defined [27–30]. *S. enterica hilD* is positively influenced by Hfq and likely interacts with the 3′ UTR of *hilD* [31,32]. One potential mechanism for positive regulation by HilD is competitive inhibition with the RNA degradosome, which as mentioned above targets the 3′ UTR for degradation (Fig. 2A) [23].

In addition to effects on mRNA stability, Hfq can also influence translation. Spot 42 is an Hfq-dependent sRNA that negatively regulates T3SS1 in *V. parahaemolyticus* [33]. Hfq promotes base pairing between Spot 42 and the 5′ UTR of vp1682 (encoding a T3SS chaperone) to decrease vp1682 translation (Fig. 3A). Because Vp1682 is required for secretion of the T3SS effector Vp1680, an *hfq* mutant results in reduced cytotoxicity of *V. parahaemolyticus* towards host cells [33].

The role of Hfq in the pathogenic *Yersiniae* appears variable and seems different in each species. Whereas Hfq is dispensable for T3SS production in *Y. enterocolitica* [34,35], Hfq positively regulates T3SS in *Y. pseudotuberculosis* and *Y. pestis*. LcrF is the AraC/XylS activator of T3SS in *Y. pestis*. In an *hfq* mutant *lcrF* transcript levels are elevated suggesting involvement of an Hfq-dependent sRNA with negative effects on *lcrF* mRNA stability. One candidate is Ysr141, an sRNA that maps to the virulence plasmid encoding the T3SS [36].

Synthesis of Transcription Factors

Temperature effects on translation—The first recognized involvement of post-transcriptional T3SS control was in *Yersinia pestis* where efficient translation of LcrF was observed only at the permissive temperature of the mammalian host (i.e., 37°C) [37]. Ensuing studies ultimately identified a thermolabile stem-loop structure in the mRNA that was located immediately upstream of the *lcrF* coding region. The stem-loop structure functions as an RNA thermometer by preventing ribosome recruitment at the non-permissive temperature but is sufficiently denatured at 37°C to allow for ribosome recruitment (Fig. 3B) [38].

CsrA RNA-binding proteins—Members of the CsrA family of small RNA-binding proteins regulate carbon metabolism, secondary metabolites, and virulence factor production in many bacteria [39]. CsrA/RsmA proteins usually inhibit ribosome recruitment and translation by binding to a GGA-containing sequence that overlaps the ribosome-binding site on target mRNAs [40]. *S. enterica* CsrA inhibits SPI1 expression by binding to the *hilD* mRNA and reducing HilD translation and mRNA stability (Fig. 1C) [41]. Although CsrA/RsmA proteins usually inhibit protein synthesis, they can also stimulate translation by enhancing mRNA stability and/or ribosome recruitment [42]. HprX is the AraC/XylS regulator of the *Xanthomonas citri* T3SS and is activated by HrpG, a sensor kinase that phosphorylates HprX [43]. RsmA positively regulates the T3SS by directly binding to the 5′ UTR of *hrpG* and increasing the mRNA half-life (Fig. 1G).

RNA helicases—RNA helicases can denature mRNA secondary structure to promote translation [44]. DEAD-Box RNA helicases, so named for a conserved Asp-Glu-Ala-Asp catalytic motif, are ATP-dependent RNA binding proteins that hydrolyze ATP to ADP and partially unfold RNA substrates [44]. *P. aeruginosa* has 7 putative DEAD box helicases, one of which (DeaD) is essential for T3SS gene expression [45]. A mutant lacking *deaD* has a specific decrease in ExsA synthesis. Although some DEAD-Box RNA helicases function as a component of the RNA degradosome, the half-life of *exsA* transcript is unaffected in a *deaD* mutant [45]. A role in translation seems more likely as purified DeaD stimulates ExsA translation *in vitro* and DeaD activity is sensitive to mutations in the DEAD motif that presumably disrupt catalytic activity (Fig. 3E) [45]. Mapping studies found that the 5' UTR of *exsA* is required for DeaD-dependent stimulation which suggests that DeaD denatures a secondary structure in the *exsA* mRNA to facilitate ribosome recruitment and translation.

Synthesis of T3SS effector proteins

Genes encoding the *yop* effectors proteins in *Y. enterocolitica* are transcribed but not translated until the injectisome is activated by calcium depletion or host cell contact [8,46]. Early genetic screens identified *yopD*, *lcrH*, and *yscM1/M2* as necessary components for repression of *yop* translation under high calcium conditions [47–49]. YopD and YscM1/2 are secreted under low calcium conditions thus providing a potential explanation for the coupling of yops translation to secretion. Despite that knowledge the actual mechanism of repression remained elusive until the Schneewind group recently reported that YopD associates with 30s ribosomal particles in an LcrH-dependent manner (Fig. 3F) [50]. Addition of YopD, LcrH, and YscM to an *in vitro* translation reaction specifically inhibits YopQ synthesis suggesting that YopD interaction with the 30s complex prevents translation initiation. The specific targeting of *yop* transcripts may involve repeated AAU sequences present in those transcripts.

CONCLUSION

Local and global regulatory mechanisms allow pathogens to integrate a variety of environmental signals into a highly integrated network that promotes efficient and effective use of the T3SS. Involvement of global regulators further promote the coordination of T3SS gene expression with other cellular functions required during host-pathogens interactions. Our current understanding of post-transcriptional control of T3SS gene expression is expanding but likely represents only a small sampling of the actually events occurring. Advancements in high-throughput sequencing and bioinformatics tools combined with multi-pronged approaches such as RNAseq, ChIPseq, ribosomal profiling, and a variety of other techniques provides significant opportunities to both discover and define additional mechanisms of post-transcriptional control.

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HIGHLIGHTS

- Post-transcriptional mechanisms integrate T3SS expression into global regulatory networks
- T3SS transcription factors are common targets of post-transcriptional control
- Involvement of RNA-binding and modifying proteins is a common feature of post-transcriptional control mechanisms

FIG 1.

Schematic representation of post-transcriptional mechanisms that regulate protein function. (A–B) Partner-switching systems allow for the coupling of T3SS gene expression to secretory activity in *P. aeruginosa* (A) and *S. flexneri* (B). Conditions where the T3SS is inactive (panels Ai and Bi) favor formation of the indicated complexes (ExsE-ExsC, ExsA-ExsD, IpaB/C-IpgC, and MxiE-OspD1-Spa15). Contact with host cells results in release of ExsE, IpaB/C, and OspD1 from the bacterial cells (panels Aii–Bii) and ensuing partner-switching by ExsC, IpgC, and Spa15. The ExsC anti-anti-activator preferentially interacts with ExsD anti-activator thereby releasing ExsA to activate T3SS gene expression (Aii). The IpgC co-activator interacts with MxiE and the Spa15/OspD1 anti-activator complex is released from MxiE to activate transcription. (C) Long chain fatty acids directly inhibit the DNA-binding activity of HilD to inhibit T3SS gene expression until cells reach the distal ileum of the the small intestines. (D) Phosphorylation of Spa47 and VirB impairs T3SS function in *S. flexneri*.

FIG 2.

Post-transcriptional effects on mRNA stability. (A) The degradosome interacts with the 3' UTR of *hliD* decreasing mRNA stability. Hfq may competitively inhibit the degradosome and increase *hliD* mRNA half-life. (B) Ribosome binding of a mini-ORF preceding *espADB* in enterohemorrhagic *Escherichia coli* protects the mRNA from degradation by the degradosome.

FIG 3.

(A) Hfq facilitates binding of the Spot42 sRNA to the 5' UTR of *vp1682* in *V. parahaemolyticus* decreasing mRNA half-life. (B) Temperature control of *lcrF* translation in *Y. pestis*. At 25°C *lcrF* leader sequence contains a complex secondary structure that is relieved at 37°C. (C) CsrA in *S. enterica* binds the 5' UTR of *hilD* blocking translation and decreasing mRNA half-life. (D) RsmA in *X. citri* binds the 5' UTR of *hrpG* increasing mRNA half-life. (E) DeaD in *P. aeruginosa* facilitates unwinding of secondary structure in *exsA* leader region promoting translation. (F) Under T3SS non-inducing conditions in *Yersinia*, YopD sequesters the 30S subunit, preventing yops translation. YopD is secreted under T3SS inducing conditions relieving the repression of the 30S subunit.