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MINIREVIEW

After the Fact(or): Posttranscriptional Gene Regulation in Enterohemorrhagic *Escherichia coli* O157:H7

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ABSTRACT To adapt to ever-changing environments, pathogens quickly alter gene expression. This can occur through transcriptional, posttranscriptional, or posttranslational regulation. Historically, transcriptional regulation has been thoroughly studied to understand pathogen niche adaptation, whereas posttranscriptional and posttranslational gene regulation has only relatively recently been appreciated to play a central role in bacterial pathogenesis. Posttranscriptional regulation may involve chaperones, nucleases, and/or noncoding small RNAs (sRNAs) and typically controls gene expression by altering the stability and/or translation of the target mRNA. In this review, we highlight the global importance of posttranscriptional regulation to enterohemorrhagic *Escherichia coli* (EHEC) gene expression and discuss specific mechanisms of how EHEC regulates expression of virulence factors critical to host colonization and disease progression. The low infectious dose of this intestinal pathogen suggests that EHEC is particularly well adapted to respond to the host environment.

KEYWORDS EHEC, enterohemorrhagic *E. coli*, pathogenesis, posttranscriptional regulation, regulation of gene expression, sRNA, virulence

Upon infecting a host, bacterial pathogens encounter many unique environments. To survive in each of these environments, pathogens must quickly alter gene expression. A major mechanism by which this occurs is through transcriptional regulation, during which transcription factors bind promoter sequences to recruit or occlude RNA polymerase to/from target promoters (1, 2). Because of the importance in global gene regulation, transcriptional regulation has been studied extensively in a variety of bacterial pathogens (see, e.g., references 3 to 7). However, transcriptional regulation is not the whole picture. Bacteria also coordinate gene expression at the posttranscriptional and posttranslational levels. These mechanisms of gene regulation provide distinct advantages to bacterial fitness by enabling bacterial pathogens to rapidly alter gene expression. For example, immediately after a stimulus is sensed by a bacterium, posttranscriptional regulation enables fast responses to adjust the expression and/or activities of proteins (8). Moreover, posttranscriptional regulation enables bacterial pathogens to fine-tune gene expression by uncoupling transcription and translation. This is beneficial because functionally related genes may be organized within an operon and thus be cotranscribed. However, a bacterium may require different levels of the resulting proteins, which can be achieved by selectively altering translation (9). Posttranslational regulation occurs mainly through protein-protein interactions and modifies the activity or amount of protein in the cell. Although posttranslational regulation may be energetically costly (by consuming ATP to degrade proteins), this type of regulation is rapid, robust, and sometimes reversible, thus allowing cells to quickly respond to stimuli (8).

Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) causes major outbreaks of food-

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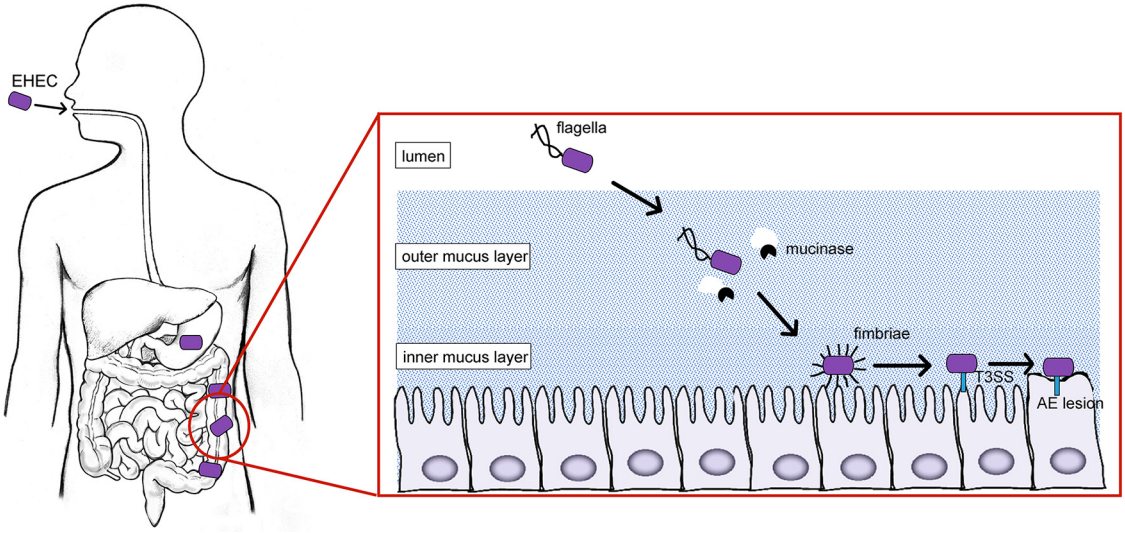

FIG 1 EHEC colonization of the colon requires precise spatiotemporal expression of genes required to move through the lumen and attach to enterocytes (see the text for details).

borne illness in developed nations. The primary reservoir of EHEC is the gastrointestinal tract of ruminant animals, and people become ill following ingestion of contaminated food or water (10). Disease consists largely of hemorrhagic colitis, but infection can lead to the development of the potentially fatal complication hemolytic-uremic syndrome (HUS) (11). HUS complicates 6 to 9% of EHEC infections overall and approximately 15% of EHEC infections in children under age 10 (12–15). Antibiotics are thought to promote expression of Shiga toxin and thereby worsen clinical manifestations of EHEC disease (16, 17). Therefore, treatment for EHEC is currently limited to supportive care, i.e., rehydration therapy (18, 19). In addition to causing severe illness, EHEC outbreaks place a heavy economic burden on the agricultural industry due to recalls of contaminated food products (20).

Following ingestion, EHEC survives and/or thrives within different host niches. For example, EHEC survives passage through the acidic stomach by expressing acid stress proteins (21–23). Subsequently, EHEC travels through the small intestine to reach the major site of colonization, the colon (24). Here, EHEC expresses distinct virulence factors that enable EHEC to move through the intestine and attach to enterocytes (Fig. 1). For example, EHEC produces mucinases and flagella to break down and penetrate through the mucus layer, respectively, and move to the epithelium (25–27). At the epithelial border, EHEC is thought to express adhesins that mediate initial adherence, which is then followed by expression of a type three secretion system (T3SS) and effectors that result in the formation of attaching-and-effacing (AE) lesions. AE lesions are characterized by effacement of microvilli and actin cytoskeletal rearrangement that results in the formation of a pedestal-like structure beneath the bacterium and intimate attachment of EHEC to enterocytes (28–30). Most of the genes involved in AE lesion formation are carried within a chromosomal pathogenicity island called the locus of enterocyte effacement (LEE) (31). The LEE contains five major operons (*LEE1* to *-5*) that encode a T3SS and effector proteins (31). EHEC's arsenal of virulence factors also includes non-LEE-encoded effector proteins that influence colonization or modulate immune responses (32–39). Finally, during infection, EHEC also produces Shiga toxin, which is responsible for the severe morbidity and mortality associated with EHEC disease (11). Significantly, ingestion of as few as 10 to 100 cells is sufficient for EHEC to establish infection (40), suggesting that EHEC employs mechanisms to quickly and precisely coordinate gene expression to survive passage through the stomach and the small intestine and then mediate attachment to the colonic epithelium. Indeed, various environmental stimuli alter the transcriptional regulation of virulence traits (25, 41, 42).

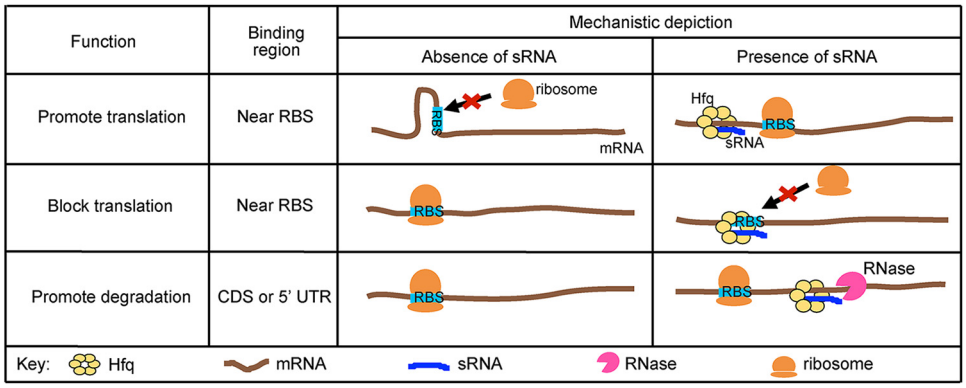

FIG 2 Canonical mechanisms of posttranscriptional regulation by *trans*-acting sRNAs. mRNAs can have secondary structures that occlude the ribosome binding site (RBS). With the help of the RNA chaperone Hfq, a small RNA (sRNA) promotes translation by binding to the target mRNA, thereby relieving secondary structures that may occlude the RBS. Alternatively, an mRNA may be efficiently translated in the absence of an sRNA, but the presence of an sRNA can inhibit translation by binding to or near the RBS and blocking ribosome access. Furthermore, an sRNA may promote degradation by recruiting RNases to the target mRNA.

Increasing evidence reveals that posttranscriptional gene regulation occurs at every stage of EHEC infection. In this review, we provide an overview of key factors involved in posttranscriptional regulation in EHEC and include relevant details of posttranslational regulation when appropriate. When they are known, we also summarize mechanisms of how expression of EHEC virulence traits is controlled via these regulatory factors.

POSTTRANSCRIPTIONAL REGULATION PLAYS A GLOBAL ROLE IN EHEC GENE EXPRESSION

Small RNAs (sRNAs) are global mediators of posttranscriptional regulation. sRNAs are typically noncoding RNAs that are 50 to 300 nucleotides in length and regulate expression of mRNAs through base pairing (43–46). sRNAs have previously been classified as *cis* or *trans* encoded; however, the term *cis* encoded can lead to some confusion, as sRNAs can be encoded within the 5′ and 3′ untranslated regions (UTRs) as well as on the noncoding DNA strand. Therefore, in this review we refer to the two classes as antisense sRNAs and *trans*-acting sRNAs. Antisense sRNAs are carried on the DNA strand opposite from the regulated gene and bind with extensive perfect complementarity. In contrast, *trans*-acting sRNAs are located in a distal location from the target mRNA(s) and usually bind to target mRNAs over short regions with imperfect complementarity, which enables a *trans*-acting sRNA to regulate multiple genes (47, 48). However, this feature frequently requires an RNA chaperone to facilitate binding interactions (43–45). In bacteria, the canonical mechanism of sRNA-mediated regulation occurs through interaction with the 5′ UTR of the target mRNA (Fig. 2), in which the sRNA sequesters the ribosome binding site (RBS) and inhibits translation (49, 50). Conversely, an sRNA may disrupt mRNA structures that occlude the RBS and thereby promote translation (51–53). Significantly, a few recent studies revealed that sRNAs can directly affect stability of a target mRNA, independently of affecting translation initiation (54–59) (Fig. 2). In these examples, the sRNA binds upstream, to the 5′ UTR or within the coding sequence (CDS) of the target mRNA (54, 57, 59).

Comparative genome analysis of 17 *E. coli* genomes (including two lab-adapted strains, one human commensal strain, and 14 pathovars [two of which were EHEC]) revealed that of approximately 4,000 to 5,000 genes per genome, only about 2,200 genes are conserved among all strains (60). These shared genes are classified as belonging to the core genome. To date, most knowledge of sRNAs in EHEC is derived from studies that were performed using nonpathogenic *E. coli* as the model organism and thus were based on core sRNAs. These studies have provided invaluable insights in understanding the extent and mechanisms of posttranscriptional regulation in the

TABLE 1 Core and EHEC-specific sRNAs that contribute to EHEC pathogenesis

General process targeted	sRNA	Target RNA	Type of regulation	Direct interaction	Reference(s)
Adherence	sRNA103	*fimZ*	Positive	Predicted	64
Global regulation	AgvB	*gcvB*	Negative	Yes	63
Iron homeostasis	AsxR	*fnsR*	Negative	Yes	63
	Esr41	*Bfr*	Negative	Yes	87
		chuA	Negative	Yes	87
		cirA	Negative	Yes	87
LEE and effectors	GlmY/Z	*LEE4*	Negative	Yes	130
		LEE5	Negative	Unknown	130
		nleA	Positive	Predicted no	64
		espFu	Positive	Predicted direct	130
	sRNA56	*espA*	Positive	Predicted no	64
	sRNA103	*espA*	Positive	Predicted no	64
	sRNA350	Unknown	Positive	Unknown	64
	Arl	*ler*	Negative	Antisense sRNA	119
	Esr055	Unknown	Positive	Unknown	147
	Spot42	*sepL*	Negative	Yes	87, 111
	RyhB	*grlRA*	Negative	Yes	112
	MgrR	*grlR*	Negative	Yes	112
Metabolism	sRNA56	*ureG*	Positive	Predicted direct	64
Motility	Esr41	*fliC*	Positive	Unknown	175

Enterobacteriaceae; however, the EHEC genome contains approximately 1.34 Mb of unique DNA sequences that are carried in pathogenicity islands (also referred to as O-islands) compared to nonpathogenic *E. coli* (61, 62). Therefore, it is likely that EHEC contains sRNAs that are not present in nonpathogenic *E. coli* and/or that the regulon of core sRNAs is more expansive in EHEC and includes regulation of virulence factors. In support of this, Tree et al. reported that the density of sRNAs encoded in O-islands is 39 sRNAs per Mb of DNA, compared to 23 sRNAs per Mb of DNA in the core genome (63). Furthermore, transcriptome sequencing (RNA-seq) experiments revealed 35 to 55 sRNAs unique to EHEC (63–65), of which only a handful have been characterized (Table 1). Additionally, RNA-seq experiments showed that core genome-encoded sRNAs regulate EHEC-specific genes, perhaps through mechanisms that are distinct from those characterized in nonpathogenic *E. coli* (Table 1). For example, the Hfq-dependent sRNAs GlmY and GlmZ provide feedback control synthesis of GlmS, an enzyme required for cell envelope synthesis (66). In EHEC, GlmY and GlmZ also regulate LEE expression as well as expression of the non-LEE-encoded effector NleA (64) (Fig. 3A). Moreover, in nonpathogenic *E. coli*, these sRNAs are characterized to work in concert to regulate gene expression (53). However, microarray analyses showed that a deletion of *glmY*

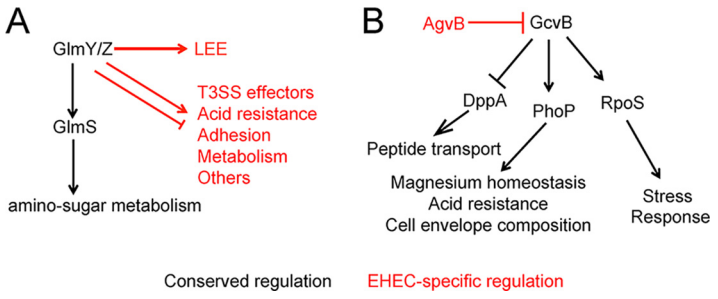

FIG 3 Comparison of the regulation and expression of core sRNAs in nonpathogenic *E. coli* and EHEC. (A) The GlmY/GlmZ regulon in nonpathogenic *E. coli* versus EHEC. (B) The EHEC-specific sRNA AgvB regulates expression of the core sRNA GcvB. Black lines indicate regulation that is conserved between nonpathogenic *E. coli* and EHEC, whereas red lines indicate regulation unique to EHEC. Arrows indicate positive regulation, and blunted lines indicate negative regulation.

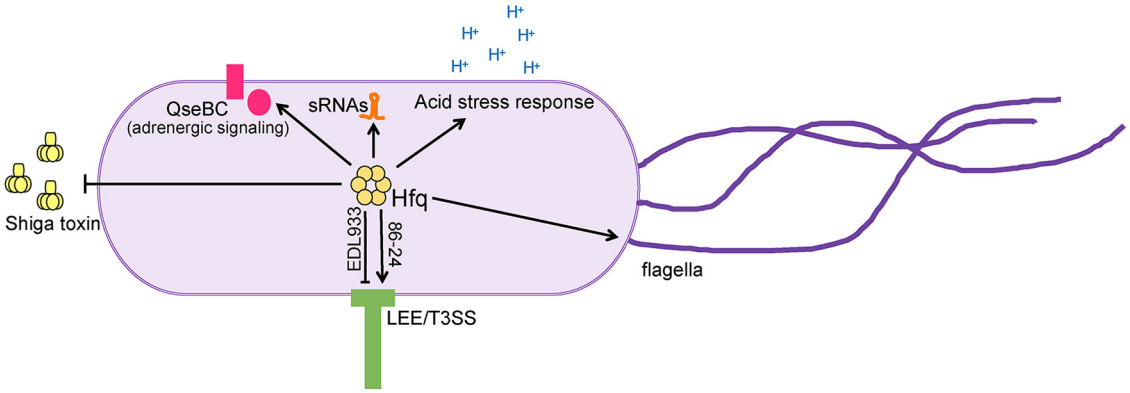

FIG 4 Hfq influences expression of virulence and regulatory factors in EHEC. Strain-dependent regulation of the LEE is indicated. Arrows indicate positive regulation, and blunted lines indicated negative regulation.

affects EHEC gene expression differently from a deletion of *glmZ* (64), suggesting that these sRNAs may also have independent functions.

The chaperone Hfq associates with many *trans*-encoded sRNAs to modulate gene expression (67, 68) (Fig. 2). Hfq is a hexameric protein that binds both sRNAs and mRNAs to facilitate interactions as well as to promote sRNA stability (69, 70). Initial, unbiased studies to examine posttranscriptional regulation in EHEC investigated Hfq-dependent changes in gene expression using microarray analyses (71–73). A deletion of *hfq* caused extensive changes in gene expression (Fig. 4), including expression of genes encoding iron acquisition, acid stress responses, regulatory proteins, flagella and motility, and sRNAs. The *hfq* deletion also resulted in increased expression of Shiga toxin (72). The importance of Hfq in EHEC virulence gene expression is further underscored in that these studies each demonstrated that Hfq affects LEE expression (71–73). Interestingly, these data demonstrated that Hfq influences LEE expression differently depending on the strain of EHEC (negatively in strain EDL933 versus positively in strain 86-24) (71–73). Strains of EHEC studied in the lab vary considerably, and the most commonly studied EHEC strains are 86-24, Sakai, and EDL933, which were isolated from patients presenting with hemorrhagic colitis (86-24 and Sakai) (61, 74) or from meat associated with an EHEC outbreak (EDL933) (75). The genomes of the EDL933, Sakai, and 86-24 EHEC strains exhibit significant variation in 27 regions of the genome in addition to differences in prophage content (76). Therefore, it is likely that these variable regions and prophages of the EHEC genomes vary in sRNA content and contribute to differences in subsequent regulation of virulence factors.

Although these studies provided an unbiased characterization of the Hfq regulon, a limitation was that the association of Hfq with a particular sRNA or specific target could not be determined (solely using microarrays). This issue is beginning to be resolved with the use of a technique called UV-induced RNA-protein cross-linking and analysis of cDNA by high-throughput sequencing (CRAC) (77). Generally, in these experiments, Hfq (or another protein) is chromosomally tagged and used as bait to capture interacting RNAs which are then cross-linked. Following Hfq purification and then subsequent digestion, the RNAs can be identified by RNA-seq. Using this method, Tree et al. identified 55 EHEC-specific sRNAs that interact with Hfq (63). Many of these EHEC-specific sRNAs are encoded within prophages and antagonize the function of other sRNAs, and thus they were termed anti-sRNAs (63). For example, one anti-sRNA, named AgvB, targets the core genome-encoded sRNA GcvB, which controls expression of genes encoding diverse functions (63, 78–81) (Fig. 3B). GcvB controls expression of the response regulator PhoP, which regulates acid resistance, magnesium homeostasis, and cell envelope homeostasis (82), as well as the sigma factor RpoS, which is involved in various stress response pathways, including acid resistance (83). GcvB also modulates expression of peptide transport systems, including decreasing expression of the dipep-

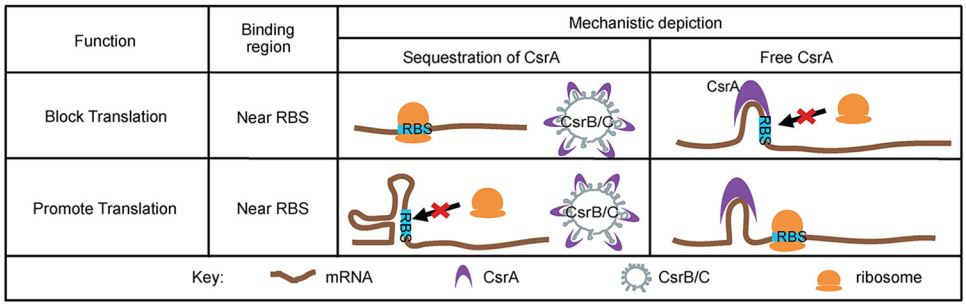

FIG 5 Canonical mechanisms of posttranscriptional regulation by CsrA. The substrate-mimetic sRNAs CsrB and CsrC sequester CsrA and limit CsrA activity. When unbound (free), CsrA binds mRNA and relieves secondary structures to promote translation. Conversely, CsrA may bind to or near the ribosome binding site (RBS) to block ribosome access and inhibit translation.

tide transporter DppA (63, 78–81). Based on data generated using a three-plasmid coexpression system, the anti-sRNA AgvB relieves GcvB repression of DppA. These data were substantiated by subsequent experiments in which DppA expression was measured in *agvB*-null strain of EHEC. Finally, the *agvB*-null strain was outcompeted by wild-type (WT) EHEC specifically when grown in mucus obtained from the terminal rectum of the bovine gastrointestinal (GI) tract (63), suggesting that anti-sRNAs are important for EHEC host colonization.

sRNAs may recruit RNases to destabilize target mRNA, and the RNase RNase E is responsible for the majority of RNA processing and turnover in *E. coli* (84–86). Based on this information, Waters et al. tagged RNase E and performed UV-cross-linking, ligation, and sequencing of hybrids (CLASH) to characterize the sRNA interactome in EHEC (87). CLASH is similar to CRAC but includes an RNA ligation step following cross-linking to purify sRNAs and the corresponding mRNA target. Using this method, Waters et al. identified nearly 2,000 sRNA-mRNA interactions, 152 unique sRNA-sRNA interactions (or "anti-sRNA"-sRNA interactions), and 320 unique sRNA-tRNA interactions (87). Whereas sRNA-mRNA and sRNA-sRNA interactions regulate gene expression, sRNA-tRNA interactions are hypothesized to serve as sponges against sRNA activity (87). As sponges, tRNAs interact with sRNAs, preventing sRNA-mRNA and sRNA-sRNA interactions. In this manner, the tRNA-sRNA interactions act to dampen sRNA activity. Although the importance of these RNA-RNA interactions in EHEC pathogenesis is not fully understood, the idea that tRNAs antagonize sRNA activity adds a new level of complexity to RNA-based regulation.

RNase E can act independently to cleave RNA molecules or in a complex called the degradosome (86). The C terminus of RNase E is a scaffold that recruits the other components of the degradosome, including the RNA helicase RhlB, the glycolytic enzyme enolase, and the polynucleotide phosphorylase (PNPase) (88–90). RhlB relieves secondary structures in mRNA (88), enolase influences RNase E localization in the cell and thus modifies RNase E activity (91), and PNPase degrades cleaved mRNA molecules (86, 92). A deletion of *pnp* (which encodes the PNPase enzyme) caused various effects on virulence gene expression in EHEC, including a decrease in expression of Shiga toxin and adherence to colonic epithelial cells *in vitro* as well as an increase in expression of some of the LEE-carried genes (93). Although this study underscored a role for PNPase in EHEC pathogenesis, the mechanism of PNPase-dependent regulation of the LEE and Shiga toxin has not yet been reported.

The CsrA (carbon storage regulatory) protein binds RNA and plays an extensive role in gene regulation in a myriad of bacteria (94). CsrA typically represses translation initiation by binding to the Shine-Dalgarno (SD) sequence and blocking ribosomal binding (95–97); however, CsrA has also been shown to activate translation and promote RNA stability (98, 99) (Fig. 5). CsrA activity is regulated by the sRNAs CsrB and CsrC. CsrB and CsrC bind multiple molecules of CsrA with a relatively high affinity and thus prevent CsrA from interacting with regulatory targets (94) (Fig. 5).

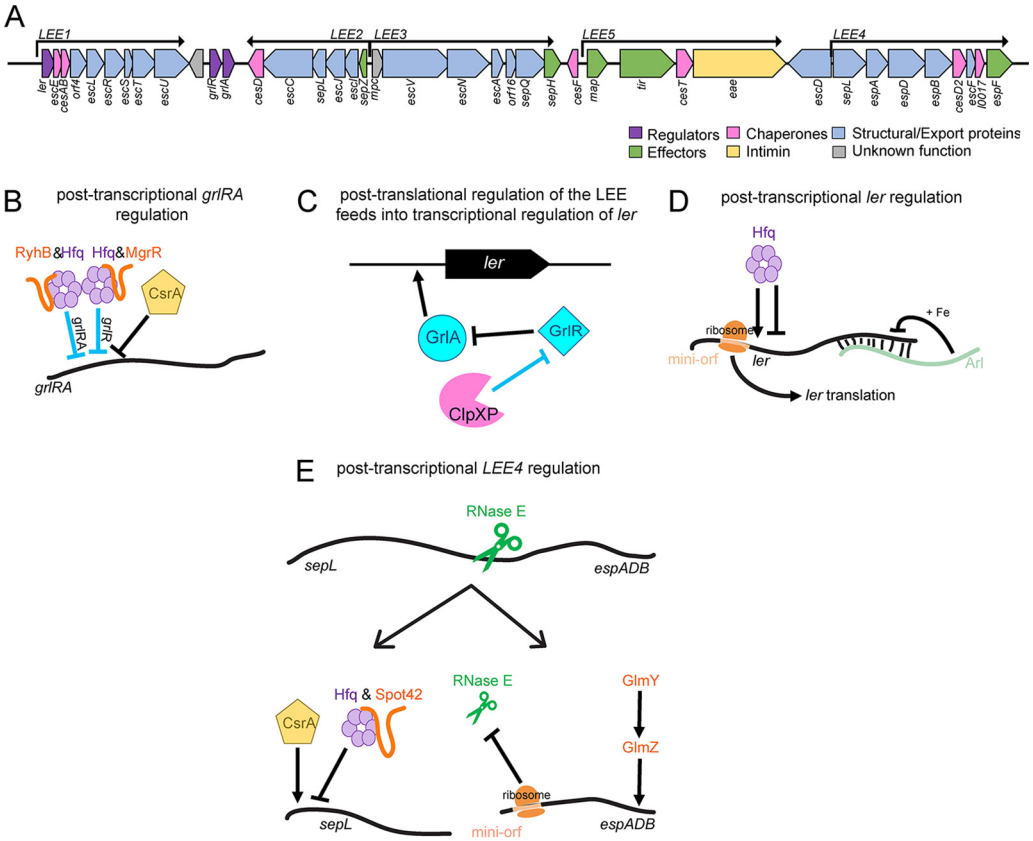

FIG 6 Posttranscriptional and posttranslational regulation of the LEE. (A) Schematic of the LEE pathogenicity island. Functions of LEE-carried genes are indicated. (B to E) Posttranscriptional or posttranslational regulation of LEE-carried genes. Black lines represent interactions shown through genetic and biochemical data, whereas blue lines represent interactions relying solely on genetic data. Arrows indicate positive regulation, and blunted lines indicate negative regulation. For details, see the associated text.

The CsrA regulon in EHEC was recently elucidated by RNA-seq (100). Similar to the case for other bacteria, CsrA plays a global role in EHEC gene expression and affects expression of the LEE as well as genes encoding motility, transport, metabolism, and signal transduction proteins (100). Mechanistic insights concerning CsrA-dependent regulation of T3S are detailed in the next section.

SPECIFIC MECHANISMS OF VIRULENCE FACTOR REGULATION

T3SS and effectors. The LEE is a horizontally acquired pathogenicity island that is perhaps the most canonical virulence factor of EHEC (101). The importance of the LEE to pathogenesis is exemplified by the requirement for colonization and pathogenesis in human *in vitro* organ culture as well as in a variety of animal models, including mice, infant rabbits, gnotobiotic pigs, and calves (24, 102–107). Proper spatiotemporal control of LEE expression is imperative to prevent energy from being wasted on premature T3SS formation as well as to evade host immune detection. The LEE carries two transcriptional regulators, Ler and GrlA. Ler is encoded by *ler*, the first gene in *LEE1*, and is the master regulator of the LEE (108). *grlR* and *grlA* are carried within an operon between *LEE1* and *LEE2* (Fig. 6A). GrlA directly binds the *ler* promoter to activate LEE expression (33, 109). However, GrlR sequesters GrlA and prevents its interaction with the *ler* promoter (109, 110). The expression and activity of Ler, GrlA, and GrlR are a hub of regulation at all levels within the cell. A recent study provided evidence that Hfq binds multiple RNA transcripts throughout the LEE, including *LEE1* and *grlRA* (111). Although the authors extensively characterized the involvement of Hfq in expression of *LEE4* (detailed below), no data describing the specific mechanism of Hfq-dependent

regulation of Ler or GrlA expression were reported in that study. However, the Hfq-dependent sRNAs MgrR and RyhB directly influence LEE expression in enteropathogenic *E. coli* (112). These sRNAs were identified following a screen that assessed the impact of core sRNAs on the activity of *grlR′-′lacZ* reporter fusions. Further characterization demonstrated that MgrR base pairs to the 5′ UTR of *grlR* to repress *grlR* expression and to activate expression of *grlA* and the LEE, whereas RyhB binds to the *grlRA* mRNA to repress translation (112) (Fig. 6B). CsrA directly binds to the *grlRA* transcript, repressing translation of *grlA* in enteropathogenic *E. coli* (EPEC) and corresponding expression of *ler* (113) (Fig. 6B). This mechanism is likely conserved in EHEC because the predicted CsrA binding site is identical (113). Finally, ClpXP, an AAA+ protease that hydrolyzes ATP to degrade substrates (114), degrades GrlR to relieve GrlA, which ultimately results in activation of LEE transcription (110, 115) (Fig. 6C).

Expression of *ler* is also controlled by *cis*-acting factors. For example, a 2-amino-acid-encoding open reading frame (ORF), termed a "minigene," promotes *ler* translation and corresponding AE lesion formation (116) (Fig. 6D). The minigene is located upstream of the *ler* start codon and affects *ler* expression in a manner that is dependent on the spacing between the minigene and the *ler* start codon (116). This is an example of translational coupling, in which translation of the minigene may increase the local concentration of ribosomes available to initiate *ler* translation or remove secondary structures that may be prohibitive for translation initiation of *ler* (117, 118); the specific mechanism of how the minigene promotes Ler expression remains to be established. Furthermore, transcription of the antisense sRNA *arl* results in repression of *ler* specifically under high-iron conditions (119) (Fig. 6D).

In addition to LEE-encoded regulatory factors, LEE genes encoding T3SS structural and functional components are posttranscriptionally regulated. For example, expression of most genes in *LEE3* is translationally coupled with the first gene in the operon, *mpc*. Disrupting *mpc* translation results in decreased expression of the entire operon to various degrees (120–122). The authors progressively mutated every SD sequence in *LEE3* and determined that of the six genes downstream of *mpc*, translation of five of the genes was dependent on translation of the gene immediately upstream (120). Mpc forms a complex with SepL and SepD. SepL and SepD are cytoplasmic proteins required for T3SS formation and function (34, 121, 123). Additionally, Mpc interacts with and promotes expression of EscA, which is required for effector secretion (33, 121). Therefore, this translational coupling may be important to ensure coordinated expression of these essential components of the basal T3SS machinery.

The *LEE4* operon encodes proteins that comprise the T3SS filament (EspA) and translocon (EspB and -D) in addition to SepL, which controls protein translocation and the chaperones CesD2 and L0017. Initial work demonstrated that different EHEC isolates expressed various levels of EspA even though levels of *LEE4* transcription in the strains were equivalent (124), which suggested a posttranscriptional mechanism of control. Indeed, subsequent work has revealed a sophisticated mechanism of *LEE4* regulation in EHEC (Fig. 6E). *sepL* is the first gene in *LEE4*, and several studies indicated that expression of the entire operon is dependent on its transcription and translation (111, 124–126) (Fig. 6E). The nascent *sepL* 5′ UTR forms a structure that sequesters the SD sequence and thereby prevents translation (111). CsrA binds the *sepL* leader sequence to expose the SD sequence and promote translation of the entire operon (111, 113). Hfq and the sRNA Spot42 also bind *sepL* mRNA and repress translation (111). The CsrA and Hfq interaction sequences on *sepL* mRNA overlap, suggesting that either CsrA or Hfq binds exclusively the transcript (111). Future work is necessary to understand whether CsrA binding to *sepL* causes additional changes in the mRNA structure (beyond exposing the RBS) that reduce CsrA binding affinity and/or enhance Hfq binding. Additionally, levels of CsrA or Hfq in the cell may affect regulation via direct competition for binding.

Additional studies have shown that RNase E also contributes to *LEE4* regulation (127, 128) (Fig. 6E). Specifically, RNase E cleaves *LEE4* transcripts between *sepL* and *espA*, which ultimately yields larger amounts of EspA than of SepL (127). RNase E degrades

transcripts containing a 5′ monophosphate (86), and thus the newly cleaved *espADB* transcript should be vulnerable to degradation. Significantly, the leader region of the *espA* mRNA carries a 6-codon "mini-ORF" that prevents further degradation of the *espADB* transcript (129). The mini-ORF contains a strong SD element that recruits ribosomes to the *espADB* leader sequence and ultimately occludes RNase E from accessing the transcript. Thus, RNase E processing of *LEE4* provides a means to differentially produce amounts of SepL and T3SS structural proteins, which are needed in smaller and larger amounts, respectively.

After reaching the required levels, expression of EspA-CesD2 needs to be turned off to allow redirection of the cell's energy to producing effector proteins. Recently, the sRNAs GlmY and GlmZ were shown to specifically destabilize the *espB-cesD2* transcript to limit further protein synthesis, without affecting stability of the *sepL* transcript (130) (Fig. 6E). Importantly, GlmY and GlmZ modulate expression of additional components important for AE lesion formation, including destabilizing the *LEE5* transcript and potentially promoting translation of the non-LEE-encoded effector EspFu/TccP (130). In conjunction with the effector Tir, EspFu/TccP recruits N-WASP and Arp2/3 to the bacterial site of attachment, which promotes effector translocation, actin rearrangement, and pedestal formation (32, 33, 35, 37, 131).

The T3SS-dependent effector NleA is an important virulence factor in EHEC. NleA contributes to host colonization and modulates host processes, including protein secretion, maintenance of the epithelial barrier, and immune responses (36, 132–134). Importantly, translation of *nleA* is precisely tuned so that NleA is not expressed until the bacterium attaches to the host cell (135). The *nleA* 5′ UTR contains a CsrA recognition sequence to which CsrA binds and inhibits translation. Significantly, the T3SS chaperone CesT antagonizes CsrA to promote NleA expression (135). Specifically, CesT binds to CsrA on a motif that overlaps the CsrA RNA binding site, which ultimately results in inhibition of CsrA-RNA interactions (136). Therefore, CesT interaction with CsrA not only promotes NleA expression but also relieves other CsrA-dependent repression within the cell to redirect gene expression upon host cell contact (135). Altogether, these findings reveal that EHEC has evolved complex and precise mechanisms to ensure proper and coordinated expression of the T3SS and effectors.

Shiga toxin. Shiga toxin (Stx) is a an AB subunit toxin that is comprised of a catalytic A subunit and a pentamer of B subunits (137, 138). The B subunits bind globotriaosylceramide (Gb3) on the surfaces of Paneth cells in the intestinal tract as well as renal epithelial cells, which induces endocytosis (139, 140). The A subunit cleaves an adenosine residue from the 28S rRNA of the 60S ribosomal subunit, inhibits protein synthesis, and ultimately triggers apoptotic host cell death (141, 142). The translocation of Stx to the kidneys is responsible for the development of HUS. Stx is encoded within a prophage, and phage-mediated cell lysis releases Stx (143). The phage-encoded sRNA 24B_1 and the EHEC-encoded enzyme PAPI influence phage entry into the lytic cycle (144, 145). These studies suggest that both phage-encoded and EHEC-encoded factors contribute to posttranscriptional regulation of the phage life cycle, which impacts Stx production and potentially disease severity. However, the implications of phage life cycle regulation for EHEC pathogenesis are unclear because these studies were performed in nonpathogenic *E. coli* strains transduced with Stx-encoding phages. Moreover, examples of direct regulation of *stx*/Stx by any posttranscriptional regulator have yet to be reported.

Adhesion and motility. EHEC carries 16 fimbrial loci in addition to other adhesins that enable attachment to abiotic and biotic surfaces and that may influence tissue tropism. The EHEC-specific sRNA Esr055 was identified in a screen for upregulated genes/sRNAs from EHEC attached to HeLa cells compared to EHEC grown in Dulbecco modified Eagle medium (DMEM) (146). Further characterization revealed that a deletion of the sRNA *esr055* increased EHEC adherence to HeLa cells. Additionally, greater numbers of the *esr055* deletion strain were recovered from the colons than from the ceca of infected mice (147). The latter findings correlate with decreased expression of

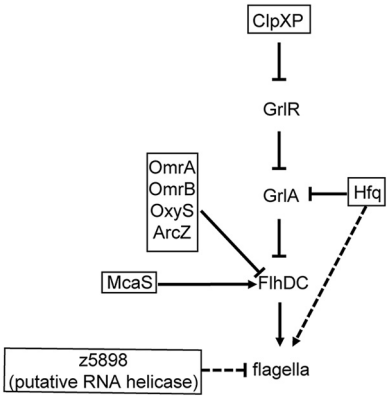

FIG 7 Posttranscriptional and posttranslational regulation of flagella. Solid lines indicate direct interactions, and dashed lines indicated indirect regulation or regulation based on genetic data. Arrows indicate positive regulation, and blunted lines indicate negative regulation.

esr055 in the colon compared to in the ileum, which suggests that Esr055 contributes to tissue tropism (147). Finally, RNA-seq experiments comparing expression of WT EHEC and the Δ*esr055* strains grown *in vitro* revealed differential expression of over 400 genes, including genes carried within fimbrial loci (147); however, direct versus indirect targets were not determined in that study.

Flagella are long whiplike surface structures that are important for motility and that also promote attachment. Flagellar expression is controlled by the master transcriptional regulator encoded by *flhD* and *flhC* (148, 149) and is also subject to complex posttranscriptional regulation (Fig. 7). In nonpathogenic *E. coli*, OxyS, OmrA, OmrB, and ArcZ negatively regulate flagellar expression by direct interaction with *flhD* (150). Furthermore, McaS positively regulates flagellar expression by binding to *flhD* (150, 151). Additionally, a deletion of the *z5898* gene, which is predicted to encode an EHEC-specific RNA helicase, resulted in decreased levels of flagellar expression and corresponding motility (152). Finally, GrlA negatively regulates transcription of *flhD* and *flhC* (110). Thus, ClpXP-dependent degradation of GrlR leads to downregulation of flagellar expression (110, 115).

Acid resistance. Although *E. coli* strains in general encode acid resistance pathways, the regulation of these pathways varies between nonpathogenic *E. coli* and EHEC (and even among EHEC isolates) (153–155). To reach the colon, EHEC must survive the acidity of the stomach. Therefore, acid resistance is considered a critical virulence factor and is thought to contribute to the low infectious dose of EHEC (23). In addition to the stomach, EHEC is exposed to weak acids in the intestine. EHEC encodes three acid resistance mechanisms to counteract these acidic environments: an oxidative system, an arginine-dependent system, and a glutamate-dependent system (21, 22). The protease ClpXP indirectly promotes expression of the glutamate-dependent acid resistance genes *gadE* and *gadX*. ClpXP rapidly degrades the sigma factor RpoS, which negatively regulates *gadE* and *gadX* transcription (156). Furthermore, the core genome-encoded antisense sRNA GadY binds to the 3′ UTR of *gadX* and thereby promotes expression (157).

Iron homeostasis. Iron is critical for the survival of most pathogens, including EHEC, because many enzymes are dependent on iron redox reactions (158). EHEC encodes several iron transporters and siderophores to acquire iron from the iron-limited intestinal environment (159). Fur (ferric uptake regulator) is an iron-sensing transcription factor and master regulator of genes encoding iron homeostasis proteins (160). As part of this, Fur activates expression of the core genome sRNA RyhB under iron-limited conditions (161). RyhB represses expression of genes encoding iron-utilizing proteins, including *sodB*, which encodes an Fe-superoxide dismutase (161, 162). Furthermore, RyhB promotes siderophore production by positively regulating expression of *shiA*,

which encodes a shikimate permease. Under iron-limited conditions, the production of shikimate allows for the synthesis of siderophores and therefore the acquisition of iron (52). RyhB and the EHEC-specific sRNA Esr41 modulate expression of the iron transport and storage proteins CirA, ChuA, and Bfr (161). RyhB negatively influences *chuA* and *bfr* expression but positively affects expression of *cirA* (161), whereas Esr41 represses translation of *chuA*, *bfr*, and *cirA* (87). Moreover, in support of the genetic data, deletion of *esr41* resulted in a growth advantage when EHEC was cultured in an iron-depleted medium (87).

SUMMARY AND OUTLOOK

Clearly, posttranscriptional regulation plays a key role in regulating traits essential for EHEC pathogenesis. The vast amount of data produced by unbiased techniques indicates that EHEC has coopted core sRNAs as well as evolved EHEC-specific sRNAs to regulate virulence. These studies have demonstrated a global role for posttranscriptional regulation; however, for the majority of sRNAs identified in EHEC, the mRNA targets and mechanisms of regulation remain to be uncovered. Moreover, most of the targeted studies have focused on regulation of the LEE, with fewer detailed reports regarding posttranscriptional regulation of other virulence factors. Posttranscriptional regulation of non-LEE virulence factors, especially Stx, warrants further study, as these pathways may represent therapeutic targets.

Furthermore, recent work has provided important insights regarding new roles for known regulatory factors, new players in posttranscriptional regulation, and novel mechanisms important for gene expression and protein function. For example, although the canonical function of Hfq is to interact with sRNAs, recent studies have shown that Hfq also functions independently of sRNAs to inhibit translation in nonpathogenic *E. coli* (163, 164). Moreover, the FinO domain protein ProQ was recently found to bind a large repertoire of sRNAs in *Salmonella enterica* serovar Typhimurium (68). In *S.* Typhimurium, ProQ not only promotes stability of a target sRNA, RaiZ, but is a component of the ternary complex that forms between the sRNA RaiZ and the 5′ UTR of the target mRNA *hupA*. The formation of the ProQ-RaiZ-*hupA* complex inhibits HU-α protein synthesis (165). Interestingly, *in vitro* biochemical data indicate that ProQ remains associated with RaiZ at the target mRNA binding site (165), whereas Hfq rapidly disassociates following sRNA-mRNA association (166). Moreover, in addition to ProQ, RaiZ also interacted with Hfq (165), suggesting that RaiZ may regulate gene expression differently depending on the associated chaperone. ProQ is conserved in EHEC, and thus similar interwoven webs of regulation will likely be uncovered in this pathogen.

RNA nucleotide modifications have also been shown to influence gene expression. For example, similar to the decapping of eukaryotic mRNAs, in bacterial transcripts, the conversion of the 5′-terminal triphosphate to a monophosphate initiates RNA decay by exposing the transcript to attack by 5′-monophosphate-dependent RNase. RppH (Nud/YgdP) is a member of the Nudix hydrolase family of enzymes. Specifically, RppH is an RNA pyrophosphohydrolase that removes 5′ phosphate from the mRNA, which results in rapid decay of the mRNA (167, 168). RppH has been shown to play an extensive role in *Helicobacter pylori* gene expression (169) and to contribute to the pathogenesis of diverse bacteria, including *E. coli* K1, *Legionella pneumophila*, and *S.* Typhimurium (170–172). Other types of mRNA modifications, such as incorporation of methylated nucleotides and/or pseudouridines, have been shown to result in premature translation termination (173).

Finally, growing evidence indicates that subcellular localization of RNAs impacts distribution of the corresponding protein products (174). This is a relatively nascent field in bacterial RNA biology, in general, and will likely have implications for understanding the function and activity of virulence factors, such as the membrane-associated T3SS or adhesins, in EHEC and other pathogens.

In summary, posttranscriptional gene regulation functions at a global level in bacteria and provides rapid and coordinated gene expression, which is essential for a pathogen to evade and/or overcome host defenses to establish infection and cause

disease. Current data underscore the importance of posttranscriptional gene regulation to spatiotemporal control of EHEC virulence factors at all stages of infection. Despite the increasing number of recent reports, it is clear that these findings are just a drop in the bucket and that there is much more to investigate. Future studies will reveal new strategies that EHEC uses to cause disease and will likely be applicable to understanding bacterial physiology and host-pathogen interactions in general.

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