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Honing the message: posttranscriptional and posttranslational control in attaching and effacing pathogens

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Abstract

Bacteria evolve their capacity to cause disease by acquiring virulence genes that are usually clustered in discrete genetic modules termed pathogenicity islands (PAI). Stable integration of PAIs into preexisting transcriptional networks coordinates expression from PAIs with ancestral genes in response to diverse environmental cues. Such transcriptional controls are evident in the regulation of the locus of enterocyte effacement (LEE), a PAI of enteropathogenic and enterohemorrhagic *Escherichia coli*. However, recent reports indicate that global posttranscriptional and posttranslational regulators, including CsrA, Hfq and ClpXP, fine-tune the transcriptional output from the LEE. Here, we highlight recent advances in understanding posttranscriptional and posttranslational regulation in attaching and effacing pathogens.

What are EPEC and EHEC?

Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) cause significant morbidity and mortality worldwide [1,2]. EPEC is a waterborne pathogen that causes diarrhea, primarily among infants, in developing countries [1]. EHEC is spread via contaminated food and water and affects both infants and adults in developed countries [1]. Certain EHEC strains harbor Shiga toxins that cause bloody diarrhea and hemolytic uremic syndrome (HUS), a disease characterized by hemolytic anemia, thrombocytopenia, and acute renal failure [2].

Upon infection, EPEC and EHEC compete with the native microflora for limited nutrients, respond to stressors from the host immune system, and migrate to sites within the small or large intestine respectively, where they colonize and cause disease [1]. They belong to the attaching and effacing (A/E) family of pathogens because they adhere intimately to intestinal cells (attachment) and promote the destruction of microvilli (effacement). Upon attachment, EPEC and EHEC recruit host cytoskeletal proteins to form characteristic actin-filled membraneous protrusions, termed pedestals, beneath themselves (Figure 1). The locus of enterocyte effacement (LEE) is a 35–42 kb pathogenicity island that encodes transcriptional regulators, a functional type III secretion system (T3SS), and various

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exported translocators and effectors. Mutational analysis has revealed that the LEE is necessary for pedestal formation and disease [1] (Figure 2).

Coordinated regulation of virulence

During infection, A/E pathogens encounter changes in pH, osmolarity, ferric nitrate [$Fe(NO_3)_3$], Ca^{2+}, temperature, quorum sensing, and bicarbonate (HCO_3^-), among others, and respond by coordinately regulating virulence gene expression in conjunction with other physiological processes [1,3]. Whereas transcriptional control of the LEE has been rigorously characterized, recent evidence indicates that posttranscriptional and posttranslational factors play key roles in fine-tuning the transcriptional output from the LEE, and in addition might integrate virulence with other physiological processes. Here we discuss such regulatory mechanisms, and highlight the physiological and evolutionary benefits of posttranscriptional and posttranslational regulation as a complement to transcriptional control. Moreover, we suggest that coordinate regulation of ancestral traits such as motility and metabolism together with virulence traits is a key determinant of morbidity in A/E pathogens.

Intrinsic transcriptional control of the LEE

The LEE is organized into five major polycistronic operons (*LEE1-LEE5*), the bicistronic operon (*grlR-grlA*) and numerous monocistronic genes (*grlA*, *escD*, *map*, *espG*, *cesF*, *rorf3*) [1,4–7] (Figure 2). Expression from the *LEE1*, *LEE2*, and *LEE3* operons and *escD* results in synthesis and assembly of a T3SS, which traverses the inner and outer membranes, and the peptidoglycan layer [1,5]. The *LEE4* operon primarily encodes the extracellular components of the T3SS including EscF, EspA, EspB and EspD. EscF forms the needle tip of the T3SS, and EspA monomers polymerize to form a hollow filament that connects EscF to the host cell membrane [8–10]. Subsequently, EspB and EspD translocate through the EspA filament and integrate into the host plasma membrane thereby forming a contiguous pore between the bacterium and the host. Effectors, including Tir, enter the host cell through the portal and mediate the formation of pedestals [1,11–15] (Figure 1a).

Under refractory conditions, the global regulator H-NS binds to *cis*-regulatory sequences located upstream of *LEE1-3*, *rorf3*, *grlR*, *grlA* and *LEE5* and inhibits transcription initiation [1,16–18] (Figure 2). H-NS-dependent transcriptional regulation is governed by diverse environmental cues including temperature and HCO_3^- ions, and it appears to be the most prominent repressor of the LEE [1,19] (Figure 2). Under conditions conducive for pedestal formation, H-NS-mediated repression of *LEE1* is relieved and transcription of *ler* is induced [1,19]. Consequently, Ler activates transcription from the other LEE-encoded transcriptional units, including *grlRA*, *LEE2*, *LEE3*, *LEE4*, *LEE5*, *map* and *espG*, primarily by competing with H-NS for overlapping binding sites [1,6,16,18,20,21] (Figure 2). The *grlRA* operon encodes a global transcriptional repressor of the LEE, GrlR, and an associated activator, GrlA [5]. Ler, GrlR, and GrlA are components of an autoregulatory loop in which GrlA further enhances transcription of *ler* [16,17,22], whereas GrlR binds to and inactivates GrlA, thereby limiting transcription of *ler* [17,23].

Besides transcriptional control, several recent studies have highlighted the importance of posttranscriptional and posttranslational mechanisms in refining gene expression from the LEE [24–33]. Importantly, many of these mechanisms were originally identified in *E. coli* [34–38], but are ubiquitously used by other bacterial pathogens including EPEC and EHEC [35,36,39].

Why control the LEE at the posttranscriptional and posttranslational levels?

There are several reasons why posttranscriptional and posttranslational controls might have evolved to complement transcriptional control. First, the operon organization of bacterial genes limits the capacity of transcription factors to differentially modulate genes within the same transcription unit. For instance, Ler activates the transcription of all the genes encoded within *LEE2*, *LEE3*, *LEE5* and *LEE4* operons without selectively affecting the expression of genes within individual transcription units [1,40]. Moreover, such a genetic organization is particularly constraining when gene products required in different stoichiometric ratios are encoded on the same transcript. For instance, the *LEE4*-encoded polycistronic transcript encodes a regulator (SepL), the structural components of a mature T3SS (EspA, EspD, EspB, and EscF), chaperones (CesD2 and L0017), and an effector (EspF) [29]. While most of the genes in the transcript contribute to T3SS in general, they are required in different concentrations; the translocators EspA, EspB and EspD are made in excess relative to the regulator SepL [29]. In EHEC, this is accomplished by posttranscriptional processing within the intracistronic segment of *sepL*, followed by the selective degradation of *sepL* and the concomitant stabilization of *espA*, *espD*, *espB* [29,31].

Second, posttranscriptional and posttranslational mechanisms allow bacteria to control gene expression over a wide dynamic range, degrading some transcripts or proteins when they are no longer needed, or retaining others in abeyance for rapid mobilization at a later time [41]. For instance, the RNA-binding protein CsrA represses translation from some transcripts by promoting their degradation (e.g. *pgaABCD*) [42] or without affecting transcript stability (e.g. *hfq*) [43]. Likewise, the adaptor protein RssB can target the alternative stationary phase sigma factor, RpoS, for degradation by the ClpXP protease or simply bind to and sequester it, consequently repressing the expression of RpoS-activated genes [44]. Thus, by affecting substrate activity, stability and/or abundance [41,44–48], posttranscriptional and posttranslational mechanisms fine-tune gene expression in a way not easily accomplished by transcriptional controls alone.

Third, posttranscriptional and posttranslational controls of gene regulation provide a means to rapidly and globally adapt to diverse environmental stimuli. The use of global regulators of ancestral processes for this purpose allows bacterial pathogens to coordinate virulence with other physiological processes. Examples of such global regulatory factors include CsrA, Hfq, DsrA, and ClpXP, whose activities are described in detail below.

Lastly, posttranscriptional regulation is energetically efficient because small RNAs (sRNAs) governing this process, such as DsrA and CsrB, or small proteins such as CsrA and Hfq, which exert much of the posttranscriptional control, can be synthesized quickly and with less energy compared to the relatively larger transcription factors [41]. In summary, posttranscriptional and posttranslational mechanisms provide a complement to transcriptional control for highly plastic regulatory responses to diverse environmental stimuli [41].

RNA-binding proteins regulate the LEE

Carbon storage regulator A (CsrA), also called RsmA, was originally identified as a repressor of glycogen biosynthesis and biofilm formation in nonpathogenic *E. coli* [49]. Since then, CsrA has been found to regulate diverse physiological processes including carbon homeostasis, quorum sensing, peptide uptake, morphogenesis of flagella, and T3SS [34,35].

CsrA is highly conserved across diverse bacteria but has been most extensively characterized in *E. coli* [34,35]. CsrA exists as a homodimer and binds to single-stranded tracts in the leader segment of transcripts containing a core motif, AGGA or ANGGA and influences messenger RNA (mRNA) stability and/or translation [34,35,50,51]. The regulatory sRNAs CsrB and CsrC contain repetitive AGGA/ANGGA tracts that sequester multiple CsrA molecules and reduce the effective concentration and activity of CsrA [51–53]. Orthologs of the Csr genes are highly conserved in A/E pathogens [24].

In EPEC, CsrA acts as an activator or repressor of the LEE in a manner that depends upon its concentration [24] (Figure 2). CsrA binds to two ANGGA motifs in the untranslated leader segment of the *LEE4* transcript, and increases the steady-state levels, likely by stabilizing the message. Additionally, CsrA also activates the expression of the inner membrane protein of the T3SS, EscD, through an intermediate regulator. As a consequence, CsrA facilitates pedestal formation [24]. By contrast, high concentrations of CsrA globally inhibit gene expression from the LEE by reducing *grlRA* transcript levels [24]. Moreover, CsrA binds to as many as three sites within the *grlRA* transcript, one of which is in close vicinity of the Shine-Dalgarno sequence, a topological feature generally conserved in repressed transcripts [34,35]. Taken together, these observations suggest that the CsrA-mediated repression of *grlRA* might result from reduced transcript stability [24].

Besides CsrA, the highly conserved RNA-chaperone Hfq has recently been identified as an important posttranscriptional regulator of the LEE in EPEC and EHEC [25,26]. Inactivation of *hfq* renders *E. coli* hypersensitive to a plethora of environmental stressors [54]. Hfq has since been recognized to play a key role in stress responses and virulence of diverse bacteria [55]. Hfq exists as a homohexamer and assumes a toroidal conformation that contains distinct proximal and distal RNA-binding surfaces [56,57]. Structural studies with Hfq from *E. coli* reveal that the proximal face binds A/U rich sequences, whereas the distal face simultaneously recognizes tandem poly-(A-R-E) tracts [56–58]. This enables Hfq to stabilize base-pairing between sRNAs and target mRNAs, which have limited complementarity, and affect transcript stability and/or translation [57].

In EHEC, Hfq represses the LEE via two pathways and consequently affects pedestal formation [25,26] (Figure 2). In the exponential phase, Hfq destabilizes the *grlRA* transcript resulting in reduced expression of GrlA. This, in turn, causes a reduction in levels of Ler, and global silencing of the LEE [25]. By contrast, in the stationary phase, Hfq-mediated repression of the LEE remains largely independent of *grlRA* and instead occurs by translational repression of Ler [25,26] (Figure 2). In both EPEC and EHEC the leader segment of the *grlRA* transcript possesses a canonical poly-(A-R-E) motif (5’-AGA AAA AGA AAG-3’), raising the possibility that the transcript binds to the distal face of Hfq. It remains to be determined whether *ler* is directly regulated by Hfq.

Besides non-catalytic RNA-binding proteins, the single-strand specific endoribonuclease RNase E also controls gene expression from the LEE of EHEC [29]. RNase E is a component of the bacterial degradosome, a multiprotein complex involved in the maturation or degradation of heterogeneous RNA species [37]. In *E. coli*, RNase E displays relaxed sequence specificity with a preference for A/U-rich transcripts [36]. Consequently, RNase E and Hfq co-regulate several transcripts antagonistically [36]. RNase E is responsible for the posttranscriptional processing of the *LEE4*-encoded *sepLespADB* transcript in EHEC [29] (Figure 2). The 5’ fragment containing *sepL* undergoes rapid degradation rendering SepL undetectable. By contrast, the 3’ segment spanning *espA* and the downstream genes remains stable [29]. Additionally, the leader segment of *sepL* possesses a noncanonical ribosome-binding site (RBS) that is highly divergent from the near-consensus RBS observed in the leader segment of *espA*. Lodato and Kaper propose that inefficient recruitment of the

ribosome to the RBS of *sepL* renders the *sepL* segment of the transcript sensitive to RNase E; by contrast, proficient binding of the ribosome to the *espA* segment and ensuing translation might sterically hinder binding by ribonucleases [29]. Such a mechanism accounts for the stoichiometric difference between the abundance of the regulator SepL and the translocators (EspA, EspB and EspD), and might also promote the transition from the export of translocators, which connect the T3SS to the host cell, to that of the effectors (e.g. Tir) [48]. Thus, in response to appropriate environmental cues, degradation of the *sepL* transcript and reduction in SepL levels could permit the secretion of effectors, which would be otherwise sequestered by SepL [29,48]. However, it remains to be determined whether posttranscriptional processing of *sepLespADB* occurs in other A/E pathogens. Furthermore, the physiological relevance of maturation of the *LEE4* transcript during synthesis of the T3SS needs to be evaluated by selective mutation of the RNase E cleavage sites in *sepL*.

sRNA-mediated regulation of the LEE

Regulatory sRNAs are ubiquitously conserved in the three taxonomic domains of life where they affect all steps of gene regulation [59]. In *E. coli*, DsrA exists as an 87-nucleotide, untranslated transcript that modulates gene expression by antisense base-pairing with its target mRNAs in the presence of the RNA-chaperone Hfq [60,61]. DsrA binds to *hns* and *rpoS* mRNAs, destabilizing the former but promoting translation of the latter [62,63]. Riboregulation of these global regulators enables DsrA to regulate responses to an array of environmental stressors [61]. In EHEC, overexpression of *dsrA* activates *ler* in an *hns*- and *rpoS*-dependent manner [27] (Figure 2). While details of this regulation have not been elucidated, it is likely that the observed phenotype results from a mechanism similar to that observed in *E. coli*. Thus, high levels of DsrA could result in loss of H-NS-mediated repression of the LEE and, consequently, activation of Ler. DsrA-mediated activation of Ler also requires a functional *rpoS* allele. Paradoxically, RpoS has also been recognized as a repressor of Ler, when DsrA is expressed in single copy, and RpoS levels are low [27,28]. However, increasing DsrA concentrations could promote translation of RpoS and lead to activation of Ler [27]. Thus, RpoS could act as a dose-dependent repressor or activator of the LEE, in a manner that depends on DsrA levels (Figure 2).

Intriguingly, the effects of DsrA are pathovar-specific, as overexpression has negligible effects on LEE gene expression in EPEC [27]. One possible explanation is that the basal levels of the DsrA message might be inherently higher in EPEC as compared to EHEC [27]. Thus, DsrA could contribute to the higher overall level of expression from the LEE of EPEC as compared to EHEC. A confounding factor in studies on EPEC and EHEC is that inactivation of *dsrA* was without effect. In this regard, riboregulators, including DsrA, often exhibit functional redundancy [41,64]. In *E. coli*, inactivation of *dsrA*, *rprA*, and *arcZ* is required to substantially reduce expression of *rpoS* under nutrient deprivation conditions [64], and the same might be necessary in EPEC and EHEC to observe physiologically relevant changes in gene expression from the LEE.

Posttranslational control of the LEE

Information on how posttranslational factors affect virulence of A/E pathogens is more limited than for posttranscriptional regulators. The ATP-dependent protease ClpXP is one of the few posttranslational regulators whose role in the virulence of EHEC is well established [28,30]. Substrates bind to ClpX in an ATP-dependent manner and are subsequently hydrolyzed in the inner cavity of the catalytic component ClpP [65]. In EHEC, ClpXP activates the transcription of *ler* in an *rpoS*- and *grlR*-dependent manner [28,30] (Figure 2). In *E. coli* K-12, the adaptor protein RssB is sufficient to target RpoS for degradation by ClpXP [44]. RssB is highly conserved in A/E pathogens suggesting that *clpXP*-mediated

activation of the LEE might result in part from the direct degradation of RpoS by ClpXP. ClpXP effects also appear to be mediated via GrlR because inactivation of *grlR* completely bypasses the requirement for functional *clpXP* [28]. The observation that the stability and abundance of GrlR is elevated in a *clpXP* mutant of EHEC raises the possibility that GrlR could be a direct substrate of ClpXP [28]. This is perhaps not surprising as intrinsic regulators of horizontally acquired genes frequently integrate into ancestral regulatory circuits, including those acting at the posttranscriptional and posttranslational level [35,55,66].

Other extra-transcriptional mechanisms affecting the LEE

Posttranscriptional regulation has also been implicated in the phenotypic plasticity of EspADB translocons evident in different EHEC strains [31]. High secretors exhibit more T3SSs on their surface than low secretors. Paradoxically, high-secretors export more translocator proteins but contain less mRNA in comparison to low-secretor strains [31]. The regulatory factors and the corresponding networks mediating this response have yet to be elucidated, except in so far as the effect appears to be at the posttranscriptional level. In EHEC, the EspA translocon also functions as an adhesin [1], and evidence in other systems suggests that adhesin levels can contribute to tissue tropism amongst related strains [66]. Such strain-specific effects raise the possibility that rewiring of posttranscriptional regulatory networks amongst EHEC strains might confer alternate sites of attachment within the intestinal tract and/or facilitate colonization of different tissues within the host.

A variety of other extra-transcriptional mechanisms have been described that regulate LEE expression, though little detailed mechanistic information is available. For example, the ribosome binding GTPase BipA induces gene expression from the LEE of EPEC and EHEC by promoting the steady-state transcript levels of *ler* [32]. Moreover, BipA is also required for the proteolysis of intimin in EPEC [32] (Figure 2). In addition, epigenetic control via the DNA-modifying enzyme DNA adenine methyltransferase (Dam) has also been implicated in regulation of the LEE in EHEC [67]. The physiological role of Dam is to methylate the adenine residue located within the tetranucleotide tract, GATC [68]. The resulting methylation pattern globally affects numerous cellular processes including general gene expression, chromosomal replication, and methyl-directed mismatch repair [67]. In EHEC, inactivation of *dam* induces gene expression from the LEE and promotes bacterial adherence and pedestal formation [33] (Figure 2). This effect appears to be mediated at the translational or posttranslational level as the abundance of the LEE-encoded proteins, but not transcripts, is dramatically elevated in the mutant [33]. It is likely that this effect on the LEE is regulated through an intermediary because Dam specifically modifies DNA [67,68].

Elucidating the posttranscriptional and posttranslational ‘virulence regulome’ of A/E pathogens

The majority of posttranscriptional and posttranslational regulators that govern the LEE are ancestral factors shared between nonpathogenic *E. coli* and A/E pathogens. Such factors affect a plethora of physiological processes. For instance, CsrA, Hfq and ClpXP control motility, metabolism and adaptation to stress in nonpathogenic and pathogenic *E. coli* [24,25,35,55,69]. The flagellum of EPEC and EHEC possesses adhesive properties and has been shown to facilitate bacterial colonization [1,70]. Moreover, the ability of EHEC to switch between glycolytic and gluconeogenic substrates is critical to its pathogenicity *in vivo* [71]. Thus, the ability of A/E pathogens to cause disease is not exclusively the result of traits conferred by horizontally acquired PAIs. Rather, a successful infection requires the contribution of both ancestral and newly acquired traits, acting in a coordinated spatiotemporal manner. Techniques that permit the genome-wide identification of regulons

and their corresponding traits are therefore important for elucidating the 'virulence regulome' of A/E pathogens. In this regard, RNA immunoprecipitation (RIP) of RNA-binding proteins, such as CsrA and Hfq, coupled to the sequencing of the bound transcripts will be instrumental in identifying the direct targets of posttranscriptional factors. Moreover, investigating transcriptome and proteome profiles of these regulators would facilitate discrimination between direct and indirect regulatory targets. At the posttranslational level, catalytically inactive variants might prove useful. For example, ClpXP variants that trap but do not degrade substrates would permit identification of direct targets. Likewise, indirect targets of ClpXP could be inferred by pairing substrate trapping with proteomic analysis. Such approaches have been successfully employed to identify regulons of posttranscriptional and posttranslational factors in nonpathogenic *E. coli* and other bacteria [55,72]. Furthermore, comparative metatranscriptomic and metaproteomic analyses could also shed light on the evolution of posttranscriptional and posttranslational regulatory networks in different A/E pathogens, as has been done for orthologous transcriptional networks in *Yersinia pestis* and *Salmonella* Typhimurium [66,73].

Lastly, even though *trans*-acting mutations are useful in identifying regulators of pathogenesis, such mutations affect several biological pathways, of which only some contribute to virulence. Therefore, construction of *cis*-regulatory mutations, for example in the binding sites of posttranscriptional and posttranslational regulators, to selectively affect particular pathways, will provide a better understanding of the relative contributions of these pathways in bacterial colonization and disease. Moreover, such analysis of *trans*- and *cis*-acting mutations should not be limited to *in vitro* studies, because predictions based on *in vitro* assays might not translate *in vivo*. This is particularly true for posttranscriptional regulators. For example, inactivation of *hfq* induces the expression of the T3SS and virulence-associated effectors in *Shigella sonnei*, *Vibrio cholerae*, and *Pseudomonas aeruginosa* [26,74–76]. However, the *hfq* mutant of each of these pathogens is dramatically attenuated *in vivo* [55,74,75,77]. The *in vivo* pathogenicity profile of the *hfq* mutants is not unexpected because *hfq* mediates adaptation to a number of different stressors [55]. Thus, despite enhanced virulence *in vitro*, *hfq* mutants of EPEC and EHEC will likely be attenuated *in vivo* due to enhanced sensitivity to host stressors.

Concluding remarks

Posttranscriptional and posttranslational regulation significantly expand the regulatory flexibility of A/E pathogens and provide additional checkpoints to refine transcriptional output. However, our current knowledge of such regulatory mechanisms, which might be both specific to A/E pathogens and more generally applicable, is still in its infancy. Nonetheless, such modes of regulation could be key factors coordinating the expression of newly acquired virulence genes with ancient metabolic processes. Finally, a comprehensive understanding of the extra-transcriptional honing of the transcriptional output might prove essential for the development of novel therapeutic measures to effectively combat A/E pathogens (Box 1).

Box 1

Outstanding questions

- How do environmental cues affect extra-transcriptional regulators of the LEE?
- What genes comprise the virulence regulon of extra-transcriptional factors and what is the contribution of such regulation to the morbidity associated with A/E pathogens *in vivo*?

- What is the hierarchical organization of the different posttranscriptional and posttranslational regulators that govern the LEE?
- Can highly conserved extra-transcriptional factors serve as targets for broad-spectrum antibiotics?
- How have ancestral extra-transcriptional networks evolved to coordinate expression of ancient processes with recently acquired pathogenic traits during development of virulence in A/E pathogens?
- Are there posttranscriptional and posttranslational virulence factors that are specific to A/E pathogens, and do such regulators affect ancestral genes just as ancestral regulators modulate virulence genes?
- Do orthologs of extra-transcriptional regulators with established roles in the virulence of other pathogens function similarly in A/E pathogens?

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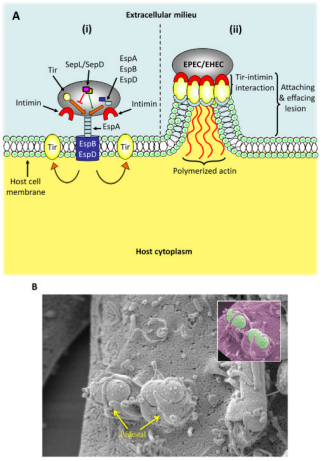

Figure 1.

Pedestal formation bv EPEC and EHEC. **(a)** Cartoon depiction of pedestal formation by EPEC and EHEC. Under inducible conditions the gatekeeper switch, composed of SepL and SepD, orchestrates the hierarchical secretion of translocators (EspA, EspB and EspD) over effectors (Tir and other effectors such as NleA, EspF and Map). While the EspADB translocon is being assembled, SepL directly binds to Tir and possibly other effectors and suppresses their export (i). After the maturation of the translocon that connects the bacterium to the host, effectors are trafficked into the host's cytoplasm via the T3SS (i). Subsequently, Tir integrates into the host plasma membrane where it interacts with its ligand, intimin, located on the outer bacterial membrane. Tir-intimin interactions promote the clustering of Tir and initiate a signal transduction cascade that leads to the conscription and subsequent polymerization of actin to form membraneous protrusions, termed attaching and effacing (A/E) pedestals, underneath adherent bacteria (ii). **(b)** Scanning electron micrographic (SEM) image of EPEC forming pedestals on infected mammalian cells. The inset shows a pseudocolored image of two EPEC bacteria (green) infecting HeLa cells (purple). SEM image courtesy of Jorge Giron.

Figure 2.

Transcriptional and extratranscriptional control of the LEE. The LEE is organized into five principal polycistronic operons (*LEE1*-5), the bicistronic operon *grlRA*, and numerous monocistronic genes. Under inducible conditions, the H-NS-mediated repression of *LEE1* is relieved and transcription of *ler* is induced. Ler, an H-NS paralog, activates transcription from the other LEE operons primarily by counteracting the H-NS-mediated repression. Expression from the LEE leads to the synthesis and consequent assembly of a T3SS that connects the bacterial cytosol to the host cytoplasm and leads to pedestal formation and ensuing disease (see text for details). The posttranscriptional factors DsrA, BipA, Hfq, and CsrA, as well as the posttranslational factor ClpXP govern gene expression from the LEE by directly or indirectly influencing the abundance of the LEE-encoded regulator, Ler. Moreover, the regulators CsrA, BipA, Dam, and RNase E exert extra-transcriptional controls on the LEE-encoded lower tier genes within the Ler regulon (see text for details). Activators and repressors have been defined based on their capacity to globally induce or silence gene expression from the LEE respectively, and consequently affect pedestal formation. Thin filled lines with blunted ends or arrowheads depict regulatory pathways where the effect is direct and/or the mechanistic basis of gene expression has been defined, whereas thin dashed lines with blunted ends or arrowheads reflect presumptive circuits for which a detailed mechanism has not been established. ++ indicates overexpression of the gene product.