

Yersinia Type III Secretion System Master Regulator LcrF

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Many Gram-negative pathogens express a type III secretion (T3SS) system to enable growth and survival within a host. The three human-pathogenic *Yersinia* species, *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*, encode the Ysc T3SS, whose expression is controlled by an AraC-like master regulator called LcrF. In this review, we discuss LcrF structure and function as well as the environmental cues and pathways known to regulate LcrF expression. Similarities and differences in binding motifs and modes of action between LcrF and the *Pseudomonas aeruginosa* homolog ExsA are summarized. In addition, we present a new bioinformatics analysis that identifies putative LcrF binding sites within *Yersinia* target gene promoters.

There are three *Yersinia* species pathogenic to humans. *Y. pestis* is the causative agent of bubonic and pneumonic plague and is transmitted through a flea vector or through airborne transmission from one mammalian host to another (1). In contrast, the enteropathogenic *Yersinia* species *Y. enterocolitica* and *Y. pseudotuberculosis* grow in the environment but can be transmitted to mammalian hosts through ingestion of contaminated food or water (1). *Y. enterocolitica* and *Y. pseudotuberculosis* cause typically self-limiting mesenteric lymphadenitis or gastroenteritis in otherwise healthy individuals but can cause a serious blood-borne infection in people with iron overload disorders such as hereditary hemochromatosis (2). In addition, sequelae following enteropathogenic *Yersinia* infection, such as erythema nodosum and reactive arthritis, have also been reported (3–5).

Human-pathogenic *Yersinia* species share a virulence plasmid, called pCD1 in *Y. pestis* and pYV in enteropathogenic yersiniae, encoding the Ysc type III secretion system (T3SS) essential for causing disease (6). These 70-kb plasmids carry dozens of T3SS structural genes and encode five or six T3SS effector proteins called Yops and their dedicated chaperones, as well as genes encoding proteins involved in regulating expression and function of the T3SS. One of these regulatory proteins, LcrF, serves as the *Yersinia* T3SS master regulator, controlling transcription of a large number of plasmid-borne genes. Several environmental cues influence expression of LcrF itself, possibly enabling *Yersinia* to control T3SS expression during transitions from one niche to another. Recent reviews have highlighted important advances in our understanding of T3SS structure and modulation of the innate immune response by T3SS effector proteins (7). In this review, we focus on factors controlling LcrF expression, the target genes LcrF regulates, and how LcrF activity influences *Yersinia* pathogenesis.

LcrF HISTORY, STRUCTURE, AND FUNCTION

It has long been appreciated that human-pathogenic *Yersinia* carrying T3SS genes requires millimolar concentrations of calcium to grow at 37°C, and this phenomenon was termed the low-calcium response, or Lcr (8, 9). The absence of calcium, in combination with a shift to 37°C, triggers secretion of T3SS effector proteins, mimicking the effect of host cell contact. *Yersinia* undergoes growth arrest during active type III secretion, explaining why calcium is required for growth at 37°C. Mechanistic explanations for how calcium ions regulate Yop secretion and why type III secretion is associated with cessation of growth *in vitro* remain unclear.

However, a number of T3SS genes were originally named for the low-calcium response, as mutations in these genes were shown to alter the Lcr phenotype (10, 11).

lcrF was first identified in *Y. pestis* by Goguen and colleagues as a gene required for thermal induction of several pCD1 genes (12). Using a similar approach, VirF, the *Y. enterocolitica* LcrF homolog, was discovered (13). Finally, while sequence analysis of *Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis* virulence plasmids revealed evidence of several rearrangements, the low-calcium response at 37°C was found to be highly conserved in all three species, suggesting the presence of LcrF in *Y. pseudotuberculosis* as well (14). In this review, we refer to the regulator as LcrF unless specifically referring to the *Y. enterocolitica* VirF homolog.

LcrF is a 30-kDa AraC-like protein that shares homology with AraC in its carboxy-terminal DNA binding region (15). AraC is well known for its role in *Escherichia coli* as a DNA binding transcriptional regulator (reviewed in reference 16). Early on, *Y. pestis* LcrF and *Y. enterocolitica* VirF were shown by the use of gel shift assays to bind directly to sequences in the *yopE* and *yopH* promoters (17). The amino-terminal domains for AraC-like proteins have been shown to be involved in self-association. Additionally, the amino-terminal domain of several AraC-like proteins binds cofactors that influence the ability of the protein to regulate transcription (16). For example, binding of the arabinose cofactor to *E. coli* AraC induces a conformational change, allowing AraC to activate transcription (16). It is thought that LcrF exists as a dimer in solution through the self-association of its amino-terminal domain; however, unlike that of *E. coli* AraC, the amino-terminal domain of LcrF has not been shown to bind additional cofactors.

As in all other AraC family transcriptional regulators, the carboxy-terminal DNA binding region of LcrF contains two helix-turn-helix (HTH) domains (18). Like all HTH domains, the recognition helix binds specific DNA residues within the major

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groove (19). DNA binding sites of LcrF and its homologs have been experimentally investigated in a number of studies (15–17, 20, 21). The first study, using DNase protection assays performed on promoters of genes activated by LcrF, suggested a common DNA binding motif, TTTT₆AGYcTgTat (capital letters represent more highly conserved residues, and Y stands for C or T) (17). Wattiau and Cornelis identified this “half-site” upstream of several T3SS genes. However, these proposed LcrF binding sites were highly variable in terms of distance from the transcriptional start site, directionality, and distance of the half-sites from each other (17). In a more recent analysis, King et al. observed that the carboxy-terminal domain of LcrF is nearly identical to the DNA binding region of the homologous AraC-like master regulator ExsA of the *Pseudomonas aeruginosa* T3SS (18). The authors further showed that *Y. pestis* LcrF binds and activates ExsA-dependent promoters in *P. aeruginosa*. Similarly, ExsA was able to induce expression of T3SS genes in *Y. pestis* in the absence of LcrF (18). LcrF and ExsA were shown to interact with a common nucleotide sequence motif (AaAAAnwnMygrCynnmmYTGYaAk), which is also recognized by activators of T3SS genes from *Photobacterium luminescens*, *Aeromonas hydrophilus*, and *Vibrio parahaemolyticus* (W stands for A or T, M for A or C, Y for C or T, R for A or G, and K for G or T [with uppercase letters representing more highly conserved residues]) (18, 21, 22).

To reconcile these two dissimilarly presented LcrF consensus binding sites, we attempted to align the promoter regions of genes known to be controlled by LcrF. First, all previously identified LcrF binding site sequences from the *virA*, *virB*, *virC*, *yopE*, *lcrG*, and *yopH* promoters, as well as known ExsA binding sites from *P. aeruginosa*, were input into MEME motif discovery to identify a consensus motif (23). The resulting motif was subsequently used to search the virulence plasmids of the three human-pathogenic *Yersinia* species to identify all putative LcrF binding sites using FIMO (Find Individual Motif Occurrences), where motifs were called with a *P* value threshold of <0.0001 (24). Alignment of LcrF binding sites of selected genes (Fig. 1A) shows a 5'-AAAA-N₆-GNCT-N₅-TGANA-3' motif located 20 to 21 bp upstream of the predicted -10 TATA box for most genes. This consensus motif is similar to that of ExsA described above. Sequences containing these motifs 20 to 21 bp upstream of the -10 TATA box overlap well the regions found experimentally to bind LcrF or ExsA (Fig. 1A, solid underlines) (15–18, 20, 21). In the case of the *yopH* and *ycE* genes, a second 5'-AAAA-N₆-GNCT-N₅-TGANA-3' motif was found further upstream and coincided with regions previously shown to be weakly bound by LcrF (Fig. 1A, dashed underlines) (17).

A closer look at the *yopE* promoter revealed surprising features that required clarification. A putative LcrF binding site within the *yopE* promoter was not located 20 to 21 bp upstream of a -10 TATA box (Fig. 1B), as are the majority of putative LcrF sites, but overlapped the annotated translational start site of *yopE* (Fig. 1A). The -10 region of *yopE*, however, may be located 30 bp upstream of the translational start site, which is 12 bp upstream of the transcriptional start site (black arrow in Fig. 1B) (25). Interestingly, Wattiau and Cornelis identified two different regions further upstream from *Y. enterocolitica yopE* that were protected by VirF during DNase I footprinting (Fig. 1B in blue boxes) (17). These regions contain sequences that are the reverse complement of the 5'-AAAA-N₆-GNCT-N₅-TGANA-3' motif. Because *ycE* and *yopE* are located in close proximity but are transcribed in opposite

directions, it is possible that the two protected regions upstream of *yopE*, one strongly bound by VirF (underlined by a solid line in Fig. 1B) and the other weakly bound (underlined by dashed lines in Fig. 1B) (17), are in fact LcrF binding motifs on the reverse DNA strand, belonging to the *ycE* promoter. This LcrF binding site phenomenon of two adjacent, coregulated, but divergent genes can also be seen in the case of *virA* and *virB* (blue solid-line box in Fig. 1A). It is worth noting that some AraC-like activators require binding sites downstream of the -10 region (26, 27). For example, in the *E. coli* AraC-like activator Rns, it was found that the inverted Rns motifs upstream of target gene -10 regions and the motifs downstream of -10 regions function in synergy (26, 27). Therefore, it is possible that both the putative LcrF binding sites far upstream and those downstream of the *yopE* transcriptional start site are required for activation of the *yopE* promoter. Interestingly, *yopE* was reported to be transcribed strongly under T3SS-inducing conditions, while *ycE* was minimally transcribed (28). Because of this departure from the known characteristics of other LcrF binding sites, whether the putative LcrF binding sites within the *yopE-ycE* promoter regions are functional and, if so, how they function remain to be determined.

Putative LcrF binding sites appear in a number of locations in the virulence plasmids of *Yersinia*. As shown in Fig. 1A, a 5'-AAAA-N₆-GNCT-N₅-TGANA-3' motif was found within the *ycE*, *yopO*, *yadA*, *yopK*, *ycH*, *ycS*, and *ycC* promoters. The transcriptional dependence of *yadA* and *yopK* (homologous to *yopQ* in *Y. enterocolitica*) on LcrF and VirF was previously demonstrated in transcriptional reporter assays in *Y. pseudotuberculosis* (29) and *Y. enterocolitica* (20), respectively. It is worth mentioning that we could not find LcrF binding sites upstream of two secreted effectors, *yopJ* and *yopM*, unless the threshold for motif calling was much lower (data not shown). Note also that *ycC* and *yopK* (from *Y. enterocolitica* and *Y. pestis*) do not seem to have a consensus TATA box following the TGANA motif, although these two genes are expressed under T3SS-inducing conditions (28). In contrast, the two LcrF binding sites upstream of *virA* and *virB* (Fig. 1A) have a TATA box at a proper distance but exhibit a weak dependence on LcrF (20).

Our analysis identified three putative LcrF binding sites within *virC* (*ycsA* to *ycsL*), upstream of *ycsA*, *ycsC*, and *ycsH* (Fig. 1A). Transcriptional regulation of this region seems to be complex. Michiels et al. proposed the presence of a single operon from *ycsA* to *ycsL* in *Y. enterocolitica* (30). However, Haddix and Straley demonstrated the presence of at least two operons within the *Y. pestis virC* region, one starting upstream of *ycsA* and the other starting upstream of *ycsF* (31). The exact operon structure of the *virC* locus, as well as the LcrF binding sites important for *virC* gene expression, remains to be clarified.

Recent findings have shown that ExsA/LcrF-dependent promoters are σ^{70} promoters (32). The conserved TGANA sequence within the broader LcrF binding motif was previously mistaken for a -35 box and is thought to bind one of the two monomers of LcrF or ExsA dimers (20). Notably, the distance between the -10 box and the TGANA motif of LcrF- and ExsA-dependent genes is 21 to 22 nucleotides (nt), while the spacing seen in typical σ^{70} -dependent promoters is 17 nucleotides between the -10 and -35 boxes (32). Decreasing the distance between the -10 box and the TGANA motif from 21 or 22 nucleotides to 17 nucleotides in the ExsA-dependent *exoT* and *exsD* genes abolishes transcriptional activation of these genes, suggesting that the 21-to-22-nucleotide

basal activity of ExsA could be compensated by its positive autoregulatory feedback loop, which leads to a rapid increase of ExsA expression under inducing conditions (34). While mutations in critical nucleotides within the major groove disrupted both LcrF and ExsA DNA binding, LcrF, but not ExsA, was able to tolerate certain mutations within the consensus binding site, presumably because LcrF binds DNA as a preformed dimer and not in the sequential, ordered manner seen with ExsA monomers (18). Indeed, an LcrF mutant unable to dimerize was shown to bind to each half-site but, similarly to ExsA, was more sensitive to mutations within the binding site (18).

REGULATION OF LcrF TARGET GENES

To date, LcrF/VirF is the only characterized transcriptional activator of T3SS genes in pathogenic *Yersinia*, and all known LcrF target genes are carried on pCD1/pYV. Several groups have shown transcriptional activation or direct binding of LcrF to the promoter regions of the *yopE*, *yopH*, *yadA*, and *ylpA* genes as well as of the *yopBD-lcrGVH* and *virC* operons, which encode T3SS effector, regulatory, and structural proteins as well as the YadA adhesin and the YlpA lipoprotein (17, 20, 29, 30, 35–37).

Several reports have suggested that regulation of T3SS genes can be mediated by proteins antagonizing LcrF (38, 39). Darwin and colleagues identified YtxR as a global transcriptional regulator that is conserved in all human-pathogenic *Yersinia* species. Overexpression of YtxR rendered *Yersinia* defective in secretion of Yops into the bacterial culture supernatant (38). Using a DNase footprinting approach, the authors showed that YtxR protected specific regions in the *yopE-sycE* and *yopH* promoters that overlapped known LcrF binding sites (denoted by green lines in Fig. 1A), suggesting that YtxR competes with LcrF for binding to T3SS gene promoters (38). While YtxR may introduce an additional layer of regulation to T3SS gene expression, the environmental conditions under which YtxR is expressed have yet to be elucidated (38).

Recently, Li et al. proposed another model in which the pYV-encoded regulatory protein LcrQ inhibits LcrF activity (39). *Y. pseudotuberculosis* LcrQ was first discovered as a gene required for calcium dependence at 37°C and was shown to be secreted by the T3SS, relieving repression of Yop expression when the concentration of LcrQ in the bacterial cytoplasm decreased as a result of active secretion (40, 41). The *Y. enterocolitica* LcrQ orthologs YscM1 and YscM2 were subsequently suggested to control Yop expression in combination with pYV-encoded factors other than VirF (42). Indeed, the T3SS YopD translocon protein and its LcrH/SycD chaperone are thought to cooperate with LcrQ or YscM1/YscM2 to negatively control T3SS gene expression (43–47). LcrQ has no obvious DNA binding domain and does not bind Yop promoter regions, as has been shown for LcrF and YtxR (39). In fact, it has been suggested that *Y. enterocolitica* YscM1 and YscM2, in cooperation with the YopD-LcrH complex, bind to the 5' untranslated regions of Yop mRNAs to inhibit translation (46). However, Li et al. proposed that *Y. pseudotuberculosis* LcrQ inhibits LcrF activity until the T3SS is assembled and LcrQ is secreted out of the cell. The authors showed that overexpression of LcrF mimicked the secretion profiles of a Δ *lcrQ* mutant. This suggested that the LcrF/LcrQ ratio may be important for activating T3SS gene transcription, but they could not detect a direct interaction of LcrF and LcrQ (39). Moreover, previous data indicated that *Y. enterocolitica* YscM1 was not capable of inhibiting VirF activity on the *yopH* promoter in the absence of other pYV-encoded factors

(42). Therefore, how LcrQ and YscM1/YscM2 repress T3SS gene expression in pathogenic *Yersinia* remains to be clarified.

TRANSCRIPTIONAL CONTROL OF LcrF

Thermoregulation of *lcrF* transcription and *ymoA*. There are several lines of evidence showing that transcription of *lcrF* is regulated by temperature. The first observations were made with *Y. enterocolitica*, in which induction of a *virF-cat* fusion, and a substantial increase in the abundance of the *virF* transcript, was detected upon temperature upshift (15, 48). A comprehensive expression analysis revealed that the *lcrF* gene of *Y. pseudotuberculosis* is transcribed from a σ^{70} -dependent promoter located upstream of the *yscW* gene (named *virG* in *Y. enterocolitica*), which in turn is located 124 bp upstream of the *lcrF* coding sequence (Fig. 2). Temperature-dependent *yscW-lcrF* transcription from this promoter is controlled by the nucleoid-associated YmoA protein, which shows homology to the Hha protein of *E. coli* (49). YmoA (for “*Yersinia* modulator”) was identified in a search for chromosomal insertion mutants of *Y. enterocolitica* transcribing *virF* and, hence, VirF-dependent *yop* and *yadA* genes at low temperatures (48). Mutations in *ymoA* led to increased *virF* and *yop* expression at 25°C and decreased expression at 37°C compared to that in the control strain, although *ymoA* mutants still induced expression of *virF* and *yop* upon temperature shift (48). Elevated expression of a *yscW-lacZ* fusion in a *ymoA*-deficient *Y. pseudotuberculosis* strain further suggested that YmoA represses *yscW-lcrF* transcription from a promoter located 264 nt upstream of the start codon of *yscW* (49) (Fig. 2). As YmoA dependency was lost when sequences downstream of the *yscW* transcriptional start site were deleted but was maintained when the regulatory region upstream of the *yscW* promoter was removed, it is assumed that YmoA influences *lcrF* expression via sequences located downstream of the *yscW* promoter (49). However, even high concentrations of purified YmoA homodimers were unable to interact specifically with the *yscW* regulatory region, indicating that an additional factor contributes to YmoA-mediated repression of *yscW-lcrF* transcription at moderate temperatures (15°C to 30°C) (49).

The small size of YmoA, its unusual high number of charged amino acid residues, and its influence on the fragility of the chromosomal DNA suggested that YmoA is a histone-like protein involved in chromosome structure similarly to histone-like nucleoid-structuring (H-NS) protein and controls *lcrF* expression through temperature-induced changes in DNA topology (48, 50, 51). Domains of members of the Hha/YmoA protein family have a striking similarity to the oligomerization domain of the H-NS nucleoid-structuring protein and its paralogs and were shown to interact specifically with different enterobacterial H-NS proteins (52, 53). Band shift analyses performed with *yscW* promoter fragments demonstrated that YmoA copurified with H-NS was able to interact specifically with sequences located downstream of the *yscW* promoter (Fig. 2) (49). Therefore, heterocomplex formation of YmoA with H-NS seems responsible for the thermoregulation of the *yscW-lcrF* operon. Interestingly, H-NS alone is also able to interact with *yscW* promoter fragments (49). In this context, it would be important to know how H-NS homodimers and H-NS/YmoA heterodimers differ in their abilities to interact with the *yscW* promoter region and how this influences expression of the *yscW-lcrF* operon in response to temperature. Several independent attempts to construct an *hns*-deficient mutant in *Yersinia* to

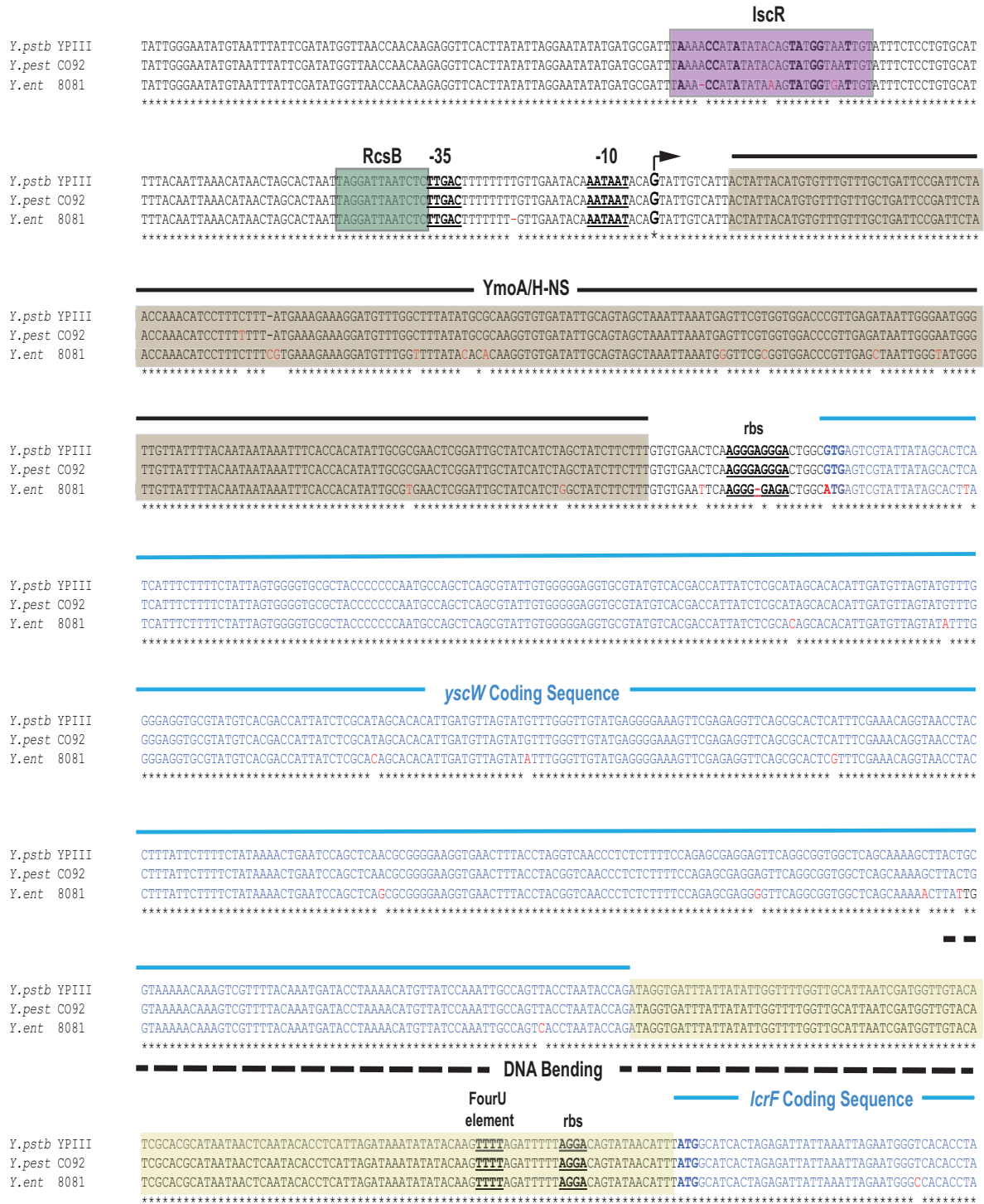


FIG 2 Regulatory elements encoded within the *yscW-lcrF* sequence. Nucleotide sequences of the *yscW-lcrF* promoter regions for *Y. pestis* C092, *Y. enterocolitica* 8081, and *Y. pseudotuberculosis* YPIII were aligned using ClustalW2. Nucleotides whose sequences are not identical are marked in red, while the conserved nucleotides are indicated by asterisks. The identified binding sequence for IscR is marked in purple, and critical residues required for IscR binding within this motif are in bold (28, 64). The binding site for RcsB is marked in green (71). The region experimentally determined to be involved in YmoA/H-NS binding, marked in brown, is downstream of the transcriptional start site (49). The sequence encoding the RNA thermometer is marked in yellow and is encoded within the intergenic region between *yscW* and *lcrF* (49). The DNA bending region identified within the *yscW-lcrF* operon is denoted with a dotted line and is within the intergenic region between *yscW* and *lcrF* (59). The -10 and -35 boxes, the transcriptional start site, the ribosome binding sites (rbs) upstream of the *yscW* and *lcrF* coding sequence, and the fourU element in the intergenic region between the *yscW* and *lcrF* coding regions are marked in bold (49). All coding sequences are marked in blue.

address this issue failed, indicating that H-NS is essential for the biological fitness of members of this genus (54, 55).

It is also quite reasonable that the DNA binding abilities of H-NS/H-NS and H-NS/YmoA complexes are differentially influenced by temperature-induced topological changes of the promoter and/or intrinsic conformational alterations of the regulatory proteins (56, 57). In fact, it has been reported that the DNA topology of the virulence plasmid undergoes a conformational change upon upshift to 37°C, leading to significant derepression of *virF-lcrF* expression (15, 30, 58, 59). Rohde et al. observed that *Y. enterocolitica* mutants resistant to the DNA gyrase inhibitor novobiocin constitutively expressed Yops, similarly to a $\Delta ymoA$ mutant (59). Using a two-dimensional (2-D) gel-based assay, the authors identified several DNA-intrinsic bends in the pYV plasmid. Interestingly, the presence of a DNA bend within the intergenic region between the *yscW* and *virF* genes was identified (Fig. 2). This bend was shown to melt at 37°C, suggesting that this intrinsic bend could potentially inhibit transcription of *virF* at noninducing temperatures (59).

Lastly, YmoA of *Y. pestis* and *Y. pseudotuberculosis* is subject to proteolysis by the Lon and ClpP proteases at 37°C but not at common environmental temperatures between 15°C and 30°C (49, 57). As a result, YmoA-mediated repression of the *yscW-lcrF* operon is rapidly eliminated at 37°C to induce the T3SS, but the repression effect remains present at all temperatures in a *clpP-lon* deletion mutant (57). Nevertheless, LcrF synthesis is still significantly enhanced in a *ymoA*-deficient strain at host body temperature compared to 25°C, indicating the importance of thermally induced DNA bending, the RNA thermometer (see below), and, possibly, H-NS homodimers.

Cross-regulation with flagellar system. Many *Yersinia* flagellar genes, including the alternative flagellum-specific σ^{28} sigma factor encoded by *fliA*, are strictly controlled by temperature. In contrast to T3SS genes, they are upregulated only at moderate temperatures and repressed at body temperature (60). *Yersinia* likely utilizes flagellar motility in the environment at temperatures under 37°C and does not require the T3SS prior to transmission into a mammalian host. This suggests an inverse regulation of flagellar and T3SS genes, and some evidence exists that σ^{28} /FliA is crucial for this process (61). Through microarray analysis comparing the *Y. enterocolitica* wild-type strain and a $\Delta fliA$ mutant, several pYV-borne genes such as *virF* were found to be upregulated in the $\Delta fliA$ mutant at 25°C (61). Furthermore, a putative binding site for FliA was identified in the *virF* promoter (61), suggesting that FliA binds to the *virF* promoter to repress transcription under temperature conditions that induce flagellar expression and assembly (61). However, we could not identify the putative FliA site described by Horne and Prüss (61) within the *virF* upstream region either by scanning for the exact FliA binding site sequence suggested by the authors or by using known FliA binding sites to make a motif model to search against the *Y. enterocolitica* genome (unpublished observations). While a discernible FliA motif may not be present in the *virF* promoter region in the current *Y. enterocolitica* genome assembly, the discrepancy might be due to differences between the current genome assembly and the one used in Horne and Prüss. Interestingly, *Y. pseudotuberculosis* bacteria lacking the RNA chaperone Hfq are hypermotile but defective in type III secretion (62). Thus, further studies are needed to identify the mechanism(s) involved in maintaining this inverse relationship between flagellar motility and T3SS expression.

IscR. It has been recently suggested that LcrF might be affected by environmental signals other than temperature. Through a forward genetic screen for modulators of the *Y. pseudotuberculosis* T3SS, the iron-sulfur cluster coordinating transcription regulator IscR was identified as important for type III secretion and *Yersinia* virulence (28). IscR is a global transcriptional regulator that has been extensively characterized in *E. coli* (63). The ability of IscR to modulate transcription of target genes depends on coordination of a [2Fe-2S] cluster, and IscR is an active transcription factor in both the apo-IscR and holo-IscR forms (63–65). This is due to the ability of IscR to recognize two separate DNA binding motifs: type I motifs are bound by holo-IscR, while type II motifs are recognized by both apo-IscR and holo-IscR (63, 64, 66). In *Y. pseudotuberculosis*, it was shown that IscR binds to a type II motif within the *lcrF* promoter (Fig. 2) (28), suggesting that IscR controls transcription of *Yersinia* type III secretion directly. Furthermore, this motif within the *lcrF* promoter is 100% conserved between *Y. pseudotuberculosis* and *Y. pestis* species and contains all nine residues found to be critical for IscR binding in *E. coli* (64). While the IscR binding site is not 100% conserved in *Y. enterocolitica*, the nine residues that were found to be critical for IscR binding are conserved (Fig. 2, marked in bold). This indicates a possible mechanism of T3SS gene regulation that is conserved among the three pathogens. It has been suggested that oxidative stress and oxygen limitation (as a result of Fe-S cluster damage) as well as iron availability influence the apo-IscR/holo-IscR ratio and that these environmental signals may affect IscR expression and activity (67–70). Therefore, while it has not yet been demonstrated, oxidative stress, oxygen limitation, or iron availability may influence expression of LcrF and the T3SS.

RcsB and CpxR in response to extracytoplasmic stress. A recent study reported that T3SS/*yop* expression in *Yersinia* is regulated by the Rcs phosphorelay system—a complex signaling pathway used by members of the *Enterobacteriaceae* to adapt their cellular physiology, biofilm and capsule formation, and motility in response to perturbations in external or surface-associated processes, e.g., overproduction of envelope components, osmotic shock, or desiccation (71). Overexpression of the wild type or a constitutive active variant of the response regulator RcsB enhanced mRNA levels of LcrF as well as Yop protein expression and secretion, suggesting that RcsB influences T3SS/Yop through LcrF (71). This was confirmed by the fact that activated/phosphorylated RcsB has the capacity to bind directly to a conserved RcsB box just upstream of the –35 promoter element of the *yscW-lcrF* operon (Fig. 2). RcsB binding most likely enhances RNA polymerase binding and/or function (71).

In addition to RcsB, it has been established that a second response regulator, CpxR, of another prominent phosphorelay system that responds to extracytoplasmic stress conditions targets the *yscW-lcrF* promoter region. However, in contrast to RcsB, CpxR represses transcription of the operon, suggesting that the two regulatory components either are part of a joint regulatory cascade or are separately induced during different infection stages or in different niches (71, 72).

Effects on *lcrF* revealed by global expression analyses. To gain insight into genes expressed by *Yersinia* during septicemia, global transcription patterns of bacteria grown in human plasma were compared with those of bacteria grown in Luria-Bertani broth, a standard laboratory medium (73, 74). *Y. pestis virG* and *yscW* and *Y. pseudotuberculosis lcrF* were specifically upregulated

in human plasma at 37°C, indicating that induction of the *yscW-lcrF* operon is important for virulence during the septicemic phase of the infection. A strong upregulation of *lcrF* gene expression was also observed at various time points after nasal infection of mice with *Y. pestis* in the lungs, spleen, and liver, supporting previous studies indicating that LcrF-controlled T3SS/*yop* genes are crucial in resisting immune and inflammatory defensive responses during the development of pneumonic plague (75).

A transcriptomic study designed to identify genes under the control of the recently recognized YbeY endonuclease demonstrated that deletion of the *ybeY* gene led to an upregulation of *lcrF* and the T3SS/*yop* genes in *Y. enterocolitica* serotype O:3 under conditions in which these genes are usually repressed (i.e., at 22°C) (76). This derepression was not caused by lower YmoA levels, since the amount of *ymoA* transcript was increased in the *ybeY* mutant. Instead, the authors suggested that *lcrF* upregulation in the *ybeY* mutant was due to increased copy numbers of the virulence plasmid or to altered regulation of global regulators (e.g., Hfq, nucleoid-structuring proteins) affecting the noncoding RNA network and/or DNA supercoiling (76).

Transcriptional profiling further revealed that bacterial membrane permeability may affect expression of LcrF and the T3SS/*Yops*. Microarray analysis showed that LcrF was upregulated by ~2-fold in a Δ *rovA* *Y. pestis* mutant grown under T3SS-inducing conditions (77). This result seems surprising, as RovA is a transcriptional regulator that is upregulated at 25°C and best known for inducing expression of invasins, an important virulence factor in enteropathogenic *Yersinia* but absent from *Y. pestis*. Through gel shift analysis, the possibility that RovA binds to the *lcrF* promoter to modulate transcription was ruled out. However, small electron-dense particles were found surrounding the Δ *rovA* mutant membrane by the use of transmission electron microscopy and the membrane permeability of the mutant was decreased compared to that of the wild type (77). The authors suggested that membrane construction is altered in the absence of RovA and that this might impact assembly and regulation of the T3SS. However, the connection between membrane integrity and LcrF has not been further explored.

TRANSLATIONAL CONTROL OF *lcrF* IN RESPONSE TO TEMPERATURE

Several early studies on temperature sensing in *Yersinia* reported that levels of protein encoded by LcrF-regulated genes such as *yopE* still change in response to temperature even under conditions in which *lcrF* transcription remains constant (37). Moreover, forced transcription of *lcrF* at low temperatures did not cause induction of LcrF-dependent genes (20). This implied that posttranscriptional mechanisms modulate LcrF synthesis and/or its specific activity in response to temperature. Hoe et al. (78) reported that *Y. pestis* LcrF is controlled at the translational level in response to temperature. This thermal control was maintained when *lcrF*, containing only 208 bp of the upstream 5' untranslated region, was transcribed by the T7 polymerase in *E. coli* (78). Based on these data, a simple model of thermal regulation of *lcrF* translation was proposed in which the presence of a short predicted thermolabile stem-loop structure, or RNA thermometer, sequesters the *lcrF* ribosome binding site (rbs) sequence and blocks translation initiation at moderate temperatures. The decreased stability or melting of this structure at higher temperatures liber-

ates the rbs sequence and allows formation of a productive mRNA complex and efficient translation (78).

A comprehensive secondary-structure prediction of the 124-bp intergenic region of *yscW* and *lcrF* performed using algorithms such as mfold and RNAfold suggested that this untranslated region of the bicistronic operon folds into two hairpin structures with a free energy of -19.67 kcal/mol (Fig. 2) (49). The second temperature-sensitive stem-loop includes a stretch of four uridines base-paired with the AGGA sequence of the rbs (49). This short motif with its simple design is referred to as a fourU element. It was first discovered in the *agsA* (aggregation suppression A) heat shock gene in *Salmonella enterica* serovar Typhimurium and bears resemblance to other potential fourU elements identified in the 5' untranslated region of the *groES* and *dnaJ* heat shock genes of *Staphylococcus aureus* and *Brucella melitensis* (79). The presence of small loops and several noncanonical base pairs coupled with a network of weak hydrogen bonds, which facilitate liberation of the *lcrF* rbs, argued for a thermolabile RNA structure prone to melting within a physiological temperature range (49).

Enzymatic structural probing experiments using RNases T1 and V1 confirmed *in silico* predictions and demonstrated temperature-induced partial, but not complete, opening of the second hairpin loop (49). Existence of a thermosensing RNA element (RNA thermometer) was further confirmed by (i) *lcrF-lacZ* translational fusions, which were thermally induced when the fusion was transcribed from a temperature-independent P_{BAD} promoter, (ii) toe printing assays demonstrating that binding of ribosomes to the *lcrF* translational start site is restricted to 37°C and does not occur at 25°C, and (iii) base substitutions within the second hairpin of the thermosensing RNA element designed to stabilize or destabilize the second stem-loop (49). These stabilizing point mutations led to a "closed" conformation of the RNA element, resulting in full repression of LcrF synthesis at 37°C. In contrast, the destabilizing mutations allowed an opening of RNA structure ("open" conformation) and enhanced LcrF production at moderate and higher temperatures. Posttranscriptional control in an RNA thermometer-like fashion was further confirmed with a deletion of a stretch of nucleotides implicated in the formation of hairpin II, which completely abolished the thermosensing function of the intergenic RNA element and provoked constitutive increased synthesis of LcrF in a temperature range from 25°C to 37°C. In contrast, an RNA thermometer variant, which consisted only of the second hairpin, was more open than the full RNA thermometer and was characterized by higher LcrF levels. Nevertheless, this shortened version was still temperature inducible, indicating that the first stem-loop is not essential for thermosensing but seems to promote proper folding and/or supports the stability of the second hairpin (49).

In order to test the physiological relevance of the *lcrF* RNA thermometer and its role in *Yersinia* virulence, the pathogenicities of a closed RNA thermometer variant and an open variant of *Y. pseudotuberculosis* were compared with that of the isogenic wild-type strain in an oral mouse infection model. Despite the fact that all mice were challenged with a normally lethal dose of *Y. pseudotuberculosis*, all animals infected with *Yersinia* mutants encoding the closed RNA thermometer variant survived and showed no visible signs of infection, similarly to an *lcrF*-deficient strain, and displayed decreased colonization of the Peyer's patches, mesenteric lymph nodes, liver, and spleen (49). Intriguingly, overexpression of the T3SS/*yop* virulence program, as displayed by the open variant, was not beneficial and did not cause greater host mortality. In contrast, colonization of some host tissues was slightly re-

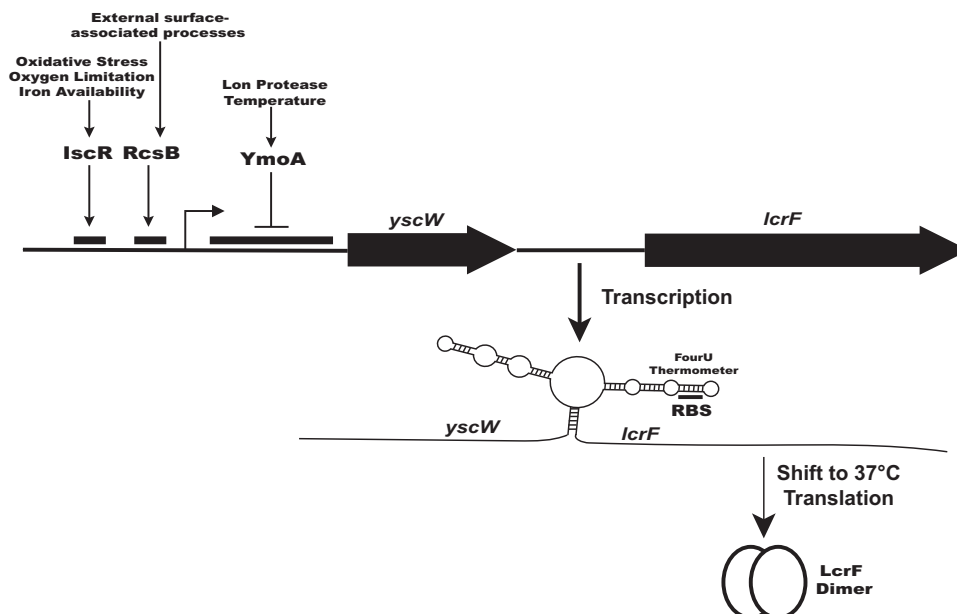


FIG 3 Multiple environmental signals control *lcrF* expression and, subsequently, T3SS expression through several distinct transcriptional and translational regulatory mechanisms. The data summarized in this review suggest that the YmoA, RcsB, and IscR regulators control transcription of *lcrF* in response to temperature, extracytoplasmic stress, iron bioavailability, oxygen tension, and reactive oxygen species. In addition, the RNA thermometer found upstream of *lcrF* allows LcrF translation only at the mammalian host body temperature, 37°C. As *Yersinia* transits from the environment or the flea vector to the mammalian host and then from localized to disseminated sites of infection, changes in temperature, iron availability, and stresses such as ROS may direct the regulatory network controlling LcrF, optimizing T3SS deployment and virulence.

duced, and the average time to death remained unchanged or was even increased by several days, most likely due to biological fitness impediments of the pathogen or increased inflammation in the host (49). This clearly illustrated that the *lcrF* RNA thermometer, all examples of which are 100% identical in all human-pathogenic *Yersinia* species, is a decisive posttranscriptional control element evolved to produce just the right amount of LcrF to promote the most ideal infection efficiency.

Apparently, both the YmoA- and RNA thermometer-mediated thermosensing mechanisms achieve a very rapid and efficient response. However, recent reports indicate that this control strategy seems to be complemented by additional regulatory modules adjusting LcrF synthesis according to host cell contact and T3SS-mediated effector translocation. In fact, the YopD translocator protein was recently found to bind to the 5' untranslated sequences of multiple T3SS/*yop* genes, including *lcrF*, which facilitates their degradation (47). The molecular mechanism of YopD-mediated repression of LcrF synthesis is still unknown, but it is possible that YopD binding to the *lcrF* transcript in the absence of host cells (i) promotes a more closed conformation of the RNA thermometer and/or (ii) accelerates the degradation of the *lcrF* transcript as a consequence of the blockage of ribosome binding and translation. Alternatively, it is possible that YopD controls expression of additional factors influencing *lcrF* transcript stability.

CONCLUSIONS

Yersinia bacteria and other T3SS-expressing pathogens have complex regulatory networks in place to control expression of T3SS genes. While the *Yersinia* T3SS master regulator LcrF was identified almost three decades ago, recent work has greatly expanded our understanding of how expression of LcrF is regulated and what environmental signals might contribute to its regulation.

YmoA, RcsB, and IscR all enhance transcription of *lcrF* (Fig. 3). As *Yersinia* experiences changes in temperature during the transition from the environment or the flea vector to the mammalian host, as well as various stresses, including iron limitation and reactive oxygen species (ROS) production during infection, sensing these environmental cues to control LcrF and T3SS expression may enable *Yersinia* to optimize T3SS deployment and virulence. While temperature has indeed been shown to contribute to *lcrF* transcription, future work focusing on expression of LcrF and T3SS genes in different host niches will enable a more complete understanding of how this T3SS master regulator facilitates optimization of T3SS expression to promote virulence.

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REFERENCES

- Putzker M, Sauer H, Sobe D. 2001. Plague and other human infections caused by *Yersinia* species. *Clin Lab* 47:453–466.
- Khan FA, Fisher MA, Khakoo RA. 2007. Association of hemochromatosis with infectious diseases: expanding spectrum. *Int J Infect Dis* 11:482–487. <http://dx.doi.org/10.1016/j.ijid.2007.04.007>.
- Bottone EJ. 1999. *Yersinia enterocolitica*: overview and epidemiologic correlates. *Microbes Infect* 1:323–333. [http://dx.doi.org/10.1016/S1286-4579\(99\)80028-8](http://dx.doi.org/10.1016/S1286-4579(99)80028-8).
- Smego RA, Frean J, Koornhof HJ. 1999. Yersiniosis I: microbiological

- and clinicoepidemiological aspects of plague and non-plague *Yersinia* infections. *Eur J Clin Microbiol Infect Dis* 18:1–15. <http://dx.doi.org/10.1007/s100960050219>.
5. Koornhof HJ, Smego RA, Jr, Nicol M. 1999. Yersiniosis. II: the pathogenesis of *Yersinia* infections. *Eur J Clin Microbiol Infect Dis* 18:87–112. <http://dx.doi.org/10.1007/s100960050237>.
 6. Cornelis GR, Boland A, Boyd AP, Geuijen C, Iriarte M, Neyt C, Sory MP, Stainier I. 1998. The virulence plasmid of *Yersinia*, an antihost genome. *Microbiol Mol Biol Rev* 62:1315–1352.
 7. Bliska JB, Wang X, Viboud GI, Brodsky IE. 2013. Modulation of innate immune responses by *Yersinia* type III secretion system translocators and effectors. *Cell Microbiol* 15:1622–1631.
 8. Kupferberg LL, Higuchi K. 1958. Role of calcium ions in the stimulation of growth of virulent strains of *Pasteurella pestis*. *J Bacteriol* 76:120–121.
 9. Higuchi K, Kupferberg LL, Smith JL. 1959. Studies on the nutrition and physiology of *Pasteurella pestis*. III. Effects of calcium ions on the growth of virulent and avirulent strains of *Pasteurella pestis*. *J Bacteriol* 77:317–321.
 10. Yother J, Goguen JD. 1985. Isolation and characterization of Ca²⁺-blind mutants of *Yersinia pestis*. *J Bacteriol* 164:704–711.
 11. Perry RD, Harmon PA, Bowmer WS, Straley SC. 1986. A low-Ca²⁺ response operon encodes the V antigen of *Yersinia pestis*. *Infect Immun* 54:428–434.
 12. Yother J, Chamness TW, Goguen JD. 1986. Temperature-controlled plasmid regulon associated with low calcium response in *Yersinia pestis*. *J Bacteriol* 165:443–447.
 13. Cornelis G, Vanootehem JC, Sluiter C. 1987. Transcription of the *yop* regulon from *Y. enterocolitica* requires *trans* acting pYV and chromosomal genes. *Microb Pathog* 2:367–379. [http://dx.doi.org/10.1016/0882-4010\(87\)90078-7](http://dx.doi.org/10.1016/0882-4010(87)90078-7).
 14. Bölin I, Forsberg A, Norlander L, Skurnik M, Wolf-Watz H. 1988. Identification and mapping of the temperature-inducible, plasmid-encoded proteins of *Yersinia* spp. *Infect Immun* 56:343–348.
 15. Cornelis G, Sluiter C, de Rouvoit CL, Michiels T. 1989. Homology between *virF*, the transcriptional activator of the *Yersinia* virulence regulon, and *AraC*, the *Escherichia coli* arabinose operon regulator. *J Bacteriol* 171:254–262.
 16. Schleif R. 2010. *AraC* protein, regulation of the L-arabinose operon in *Escherichia coli*, and the light switch mechanism of *AraC* action. *FEMS Microbiol Rev* 34:779–796. <http://dx.doi.org/10.1111/j.1574-6976.2010.00226.x>.
 17. Wattiau P, Cornelis GR. 1994. Identification of DNA sequences recognized by *VirF*, the transcriptional activator of the *Yersinia yop* regulon. *J Bacteriol* 176:3878–3884.
 18. King JM, Schesser Bartra S, Plano G, Yahr TL. 2013. ExsA and LcrF recognize similar consensus binding sites, but differences in their oligomeric state influence interactions with promoter DNA. *J Bacteriol* 195:5639–5650. <http://dx.doi.org/10.1128/JB.00990-13>.
 19. Rhee S, Martin RG, Rosner JL, Davies DR. 1998. A novel DNA-binding motif in *MarA*: the first structure for an *AraC* family transcriptional activator. *Proc Natl Acad Sci U S A* 95:10413–10418. <http://dx.doi.org/10.1073/pnas.95.18.10413>.
 20. Lambert de Rouvoit C, Sluiter C, Cornelis GR. 1992. Role of the transcriptional activator, *VirF*, and temperature in the expression of the pYV plasmid genes of *Yersinia enterocolitica*. *Mol Microbiol* 6:395–409. <http://dx.doi.org/10.1111/j.1365-2958.1992.tb01483.x>.
 21. Diaz MR, King JM, Yahr TL. 2011. Intrinsic and extrinsic regulation of type III secretion gene expression in *Pseudomonas aeruginosa*. *Front Microbiol* 2:89.
 22. Brutinel ED, Vakulskas CA, Brady KM, Yahr TL. 2008. Characterization of ExsA and of ExsA-dependent promoters required for expression of the *Pseudomonas aeruginosa* type III secretion system. *Mol Microbiol* 68:657–671. <http://dx.doi.org/10.1111/j.1365-2958.2008.06179.x>.
 23. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, Ren J, Li WW, Noble WS. 2009. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res* 37:W202–W208. <http://dx.doi.org/10.1093/nar/gkp335>.
 24. Grant CE, Bailey TL, Noble WS. 2011. FIMO: scanning for occurrences of a given motif. *Bioinformatics* 27:1017–1018. <http://dx.doi.org/10.1093/bioinformatics/btr064>.
 25. Nuss AM, Heroven AK, Waldmann B, Reinkensmeier J, Jarek M, Beckstette M, Dersch P. 2015. Transcriptomic profiling of *Yersinia pseudotuberculosis* reveals reprogramming of the Crp regulon by temperature and uncovers Crp as a master regulator of small RNAs. *PLoS Genet* 11:e1005087. <http://dx.doi.org/10.1371/journal.pgen.1005087>.
 26. Munson GP, Scott JR. 2000. Rns, a virulence regulator within the *AraC* family, requires binding sites upstream and downstream of its own promoter to function as an activator. *Mol Microbiol* 36:1391–1402.
 27. Munson GP, Holcomb LG, Scott JR. 2001. Novel group of virulence activators within the *AraC* family that are not restricted to upstream binding sites. *Infect Immun* 69:186–193. <http://dx.doi.org/10.1128/IAI.69.1.186-193.2001>.
 28. Miller HK, Kwuan L, Schwiesow L, Bernick DL, Mettert E, Ramirez HA, Ragle JM, Chan PP, Kiley PJ, Lowe TM, Auerbuch V. 2014. *IscR* is essential for *Yersinia pseudotuberculosis* type III secretion and virulence. *PLoS Pathog* 10:e1004194. <http://dx.doi.org/10.1371/journal.ppat.1004194>.
 29. China B, Michiels T, Cornelis GR. 1990. The pYV plasmid of *Yersinia* encodes a lipoprotein, YlpA, related to TraT. *Mol Microbiol* 4:1585–1593. <http://dx.doi.org/10.1111/j.1365-2958.1990.tb02070.x>.
 30. Michiels T, Vanootehem JC, Lambert de Rouvoit C, China B, Gustin A, Boudry P, Cornelis GR. 1991. Analysis of *virC*, an operon involved in the secretion of Yop proteins by *Yersinia enterocolitica*. *J Bacteriol* 173:4994–5009.
 31. Haddix PL, Straley SC. 1992. Structure and regulation of the *Yersinia pestis yscBCDEF* operon. *J Bacteriol* 174:4820–4828.
 32. Vakulskas CA, Brady KM, Yahr TL. 2009. Mechanism of transcriptional activation by *Pseudomonas aeruginosa* ExsA. *J Bacteriol* 191:6654–6664. <http://dx.doi.org/10.1128/JB.00902-09>.
 33. Brutinel ED, Vakulskas CA, Yahr TL. 2009. Functional domains of ExsA, the transcriptional activator of the *Pseudomonas aeruginosa* type III secretion system. *J Bacteriol* 191:3811–3821. <http://dx.doi.org/10.1128/JB.00002-09>.
 34. Yahr TL, Frank DW. 1994. Transcriptional organization of the trans-regulatory locus which controls exoenzyme S synthesis in *Pseudomonas aeruginosa*. *J Bacteriol* 176:3832–3838.
 35. Michiels T, Cornelis G. 1988. Nucleotide sequence and transcription analysis of *yop51* from *Yersinia enterocolitica* W22703. *Microb Pathog* 5:449–459. [http://dx.doi.org/10.1016/0882-4010\(88\)90006-X](http://dx.doi.org/10.1016/0882-4010(88)90006-X).
 36. Skurnik M, Toivanen P. 1992. LcrF is the temperature-regulated activator of the *yadA* gene of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *J Bacteriol* 174:2047–2051.
 37. Hoe NP, Minion FC, Goguen JD. 1992. Temperature sensing in *Yersinia pestis*: regulation of *yopE* transcription by *lcrF*. *J Bacteriol* 174:4275–4286.
 38. Axler-DiPerte GL, Hinchliffe SJ, Wren BW, Darwin AJ. 2009. YtxR acts as an overriding transcriptional off switch for the *Yersinia enterocolitica* Ysc-Yop type 3 secretion system. *J Bacteriol* 191:514–524. <http://dx.doi.org/10.1128/JB.01305-08>.
 39. Li L, Yan H, Feng L, Li Y, Lu P, Hu Y, Chen S. 2014. LcrQ blocks the role of LcrF in regulating the Ysc-Yop type III secretion genes in *Yersinia pseudotuberculosis*. *PLoS One* 9:e92243. <http://dx.doi.org/10.1371/journal.pone.0092243>.
 40. Pettersson J, Nordfelth R, Dubinina E, Bergman T, Gustafsson M, Magnusson KE, Wolf-Watz H. 1996. Modulation of virulence factor expression by pathogen target cell contact. *Science* 273:1231–1233. <http://dx.doi.org/10.1126/science.273.5279.1231>.
 41. Rimpiläinen M, Forsberg A, Wolf-Watz H. 1992. A novel protein, LcrQ, involved in the low-calcium response of *Yersinia pseudotuberculosis* shows extensive homology to YopH. *J Bacteriol* 174:3355–3363.
 42. Stainier I, Iriarte M, Cornelis GR. 1997. YscM1 and YscM2, two *Yersinia enterocolitica* proteins causing downregulation of *yop* transcription. *Mol Microbiol* 26:833–843. <http://dx.doi.org/10.1046/j.1365-2958.1997.6281995.x>.
 43. Kopaskie KS, Ligtenberg KG, Schneewind O. 2013. Translational regulation of *Yersinia enterocolitica* mRNA encoding a type III secretion substrate. *J Biol Chem* 288:35478–35488. <http://dx.doi.org/10.1074/jbc.M113.504811>.
 44. Williams AW, Straley SC. 1998. YopD of *Yersinia pestis* plays a role in negative regulation of the low-calcium response in addition to its role in translocation of Yops. *J Bacteriol* 180:350–358.
 45. Anderson DM, Ramamurthi KS, Tam C, Schneewind O. 2002. YopD and LcrH regulate expression of *Yersinia enterocolitica* YopQ by a post-transcriptional mechanism and bind to yopQ RNA. *J Bacteriol* 184:1287–1295. <http://dx.doi.org/10.1128/JB.184.5.1287-1295.2002>.
 46. Cambronne ED, Schneewind O. 2002. *Yersinia enterocolitica* type III secretion: *yscM1* and *yscM2* regulate *yop* gene expression by a posttran-

- scriptional mechanism that targets the 5' untranslated region of *yop* mRNA. *J Bacteriol* 184:5880–5893. <http://dx.doi.org/10.1128/JB.184.21.5880-5893.2002>.
47. Chen Y, Anderson DM. 2011. Expression hierarchy in the *Yersinia* type III secretion system established through YopD recognition of RNA. *Mol Microbiol* 80:966–980. <http://dx.doi.org/10.1111/j.1365-2958.2011.07623.x>.
 48. Cornelis G, Sluiter C, Delor I, Geib D, Kaniga K, Lambert de Rouvroit C, Sory MP, Vanooteghem JC, Michiels T. 1991. *ymoA*, a *Yersinia enterocolitica* chromosomal gene modulating the expression of virulence functions. *Mol Microbiol* 5:1023–1034. <http://dx.doi.org/10.1111/j.1365-2958.1991.tb01875.x>.
 49. Böhme K, Steinmann R, Seekircher S, Heroven AK, Berger E, Pisano F, Thiermann T, Wolf-Watz H, Narberhaus F, Dersch P. 2012. Concerted actions of a thermo-labile regulator and a unique intergenic RNA thermosensor control *Yersinia* virulence. *PLoS Pathog* 8:e1002518. <http://dx.doi.org/10.1371/journal.ppat.1002518>.
 50. Cornelis GR. 1993. Role of the transcription activator *virF* and the histone-like protein YmoA in the thermoregulation of virulence functions in *Yersinia*. *Zentralbl Bacteriol* 278:149–164. [http://dx.doi.org/10.1016/S0934-8840\(11\)80833-9](http://dx.doi.org/10.1016/S0934-8840(11)80833-9).
 51. Mikulskis AV, Cornelis GR. 1994. A new class of proteins regulating gene expression in enterobacteria. *Mol Microbiol* 11:77–86. <http://dx.doi.org/10.1111/j.1365-2958.1994.tb00291.x>.
 52. Madrid C, Nieto JM, Juarez A. 2002. Role of the Hha/YmoA family of proteins in the thermoregulation of the expression of virulence factors. *Int J Med Microbiol* 291:425–432.
 53. Nieto J, Madrid C, Miquelay E, Parra JL, Rodriguez S, Juarez A. 2002. Evidence for direct protein-protein interaction between members of the enterobacterial Hha/YmoA and H-NS families of proteins. *J Bacteriol* 184:629–635. <http://dx.doi.org/10.1128/JB.184.3.629-635.2002>.
 54. Heroven A, Nagel G, Tran HJ, Parr S, Dersch P. 2004. RovA is auto-regulated and antagonizes H-NS-mediated silencing of *invasin* and *rovA* expression in *Yersinia pseudotuberculosis*. *Mol Microbiol* 53:871–888. <http://dx.doi.org/10.1111/j.1365-2958.2004.04162.x>.
 55. Ellison DW, Miller VL. 2006. H-NS represses *inv* transcription in *Yersinia enterocolitica* through competition with RovA and interaction with YmoA. *J Bacteriol* 188:5101–5112. <http://dx.doi.org/10.1128/JB.00862-05>.
 56. Ono S, Goldberg MD, Olsson T, Esposito D, Hinton JC, Ladbury JE. 2005. H-NS is a part of a thermally controlled mechanism for bacterial gene regulation. *Biochem J* 391:203–213. <http://dx.doi.org/10.1042/BJ20050453>.
 57. Jackson M, Silva-Herzog E, Plano GV. 2004. The ATP-dependent ClpXP and Lon proteases regulate expression of the *Yersinia pestis* type III secretion system via regulated proteolysis of YmoA, a small histone-like protein. *sMol Microbiol* 54:1364–1378. <http://dx.doi.org/10.1111/j.1365-2958.2004.04353.x>.
 58. Rohde JR, Fox JM, Minnich SA. 1994. Thermoregulation in *Yersinia enterocolitica* is coincident with changes in DNA supercoiling. *Mol Microbiol* 12:187–199. <http://dx.doi.org/10.1111/j.1365-2958.1994.tb01008.x>.
 59. Rohde JR, Luan XS, Rohde H, Fox JM, Minnich SA. 1999. The *Yersinia enterocolitica* pYV virulence plasmid contains multiple intrinsic DNA bends which melt at 37 degrees C. *J Bacteriol* 181:4198–4204.
 60. Kapatral V, Olson JW, Pepe JC, Miller VL, Minnich SA. 1996. Temperature-dependent regulation of *Yersinia enterocolitica* Class III flagellar genes. *Mol Microbiol* 19:1061–1071. <http://dx.doi.org/10.1046/j.1365-2958.1996.452978.x>.
 61. Horne SM, Prüss BM. 2006. Global gene regulation in *Yersinia enterocolitica*: effect of FlhA on the expression levels of flagellar and plasmid-encoded virulence genes. *Arch Microbiol* 185:115–126. <http://dx.doi.org/10.1007/s00203-005-0077-1>.
 62. Schiano CA, Bellows LE, Latham WW. 2010. The small RNA chaperone Hfq is required for the virulence of *Yersinia pseudotuberculosis*. *Infect Immun* 78:2034–2044. <http://dx.doi.org/10.1128/IAI.01046-09>.
 63. Schwartz CJ, Giel JL, Patschkowski T, Luther C, Ruzicka FJ, Beinert H, Kiley PJ. 2001. IscR, an Fe-S cluster-containing transcription factor, represses expression of *Escherichia coli* genes encoding Fe-S cluster assembly proteins. *Proc Natl Acad Sci U S A* 98:14895–14900. <http://dx.doi.org/10.1073/pnas.251550898>.
 64. Nesbit AD, Giel JL, Rose JC, Kiley PJ. 2009. Sequence-specific binding to a subset of IscR-regulated promoters does not require IscR Fe-S cluster ligation. *J Mol Biol* 387:28–41. <http://dx.doi.org/10.1016/j.jmb.2009.01.055>.
 65. Fleischhacker AS, Stubna A, Hsueh KL, Guo Y, Teter SJ, Rose JC, Brunold TC, Markley JL, Munck E, Kiley PJ. 2012. Characterization of the [2Fe-2S] cluster of *Escherichia coli* transcription factor IscR. *Biochemistry* 51:4453–4462. <http://dx.doi.org/10.1021/bi3003204>.
 66. Rajagopalan S, Teter SJ, Zwart PH, Brennan RG, Phillips KJ, Kiley PJ. 2013. Studies of IscR reveal a unique mechanism for metal-dependent regulation of DNA binding specificity. *Nat Struct Mol Biol* 20:740–747. <http://dx.doi.org/10.1038/nsmb.2568>.
 67. Mettert EL, Kiley PJ. 2014. Coordinate regulation of the Suf and Isc Fe-S cluster biogenesis pathways by IscR is essential for viability of *Escherichia coli*. *J Bacteriol* 196:4315–4323. <http://dx.doi.org/10.1128/JB.01975-14>.
 68. Giel JL, Rodionov D, Liu M, Blattner FR, Kiley PJ. 2006. IscR-dependent gene expression links iron-sulphur cluster assembly to the control of O₂-regulated genes in *Escherichia coli*. *Mol Microbiol* 60:1058–1075. <http://dx.doi.org/10.1111/j.1365-2958.2006.05160.x>.
 69. Yeo WS, Lee JH, Lee KC, Roe JH. 2006. IscR acts as an activator in response to oxidative stress for the *suf* operon encoding Fe-S assembly proteins. *Mol Microbiol* 61:206–218. <http://dx.doi.org/10.1111/j.1365-2958.2006.05220.x>.
 70. Wu Y, Outten FW. 2009. IscR controls iron-dependent biofilm formation in *Escherichia coli* by regulating type I fimbria expression. *J Bacteriol* 191:1248–1257. <http://dx.doi.org/10.1128/JB.01086-08>.
 71. Li Y, Hu Y, Francis MS, Chen S. 2015. RcsB positively regulates the *Yersinia* Ysc-Yop type III secretion system by activating expression of the master transcriptional regulator LcrF. *Environ Microbiol* 17:1219–1233. <http://dx.doi.org/10.1111/1462-2920.12556>.
 72. Liu J, Thanikkal EJ, Obi IR, Francis MS. 2012. Elevated CpxR~P levels repress the Ysc-Yop type III secretion system of *Yersinia pseudotuberculosis*. *Res Microbiol* 163:518–530. <http://dx.doi.org/10.1016/j.resmic.2012.07.010>.
 73. Rosso ML, Chauvaux S, Dessein R, Laurans C, Frangeul L, Lacroix C, Schiavo A, Dillies MA, Foulon J, Coppee JY, Medigue C, Carniel E, Simonet M, Marceau M. 2008. Growth of *Yersinia pseudotuberculosis* in human plasma: impacts on virulence and metabolic gene expression. *BMC Microbiol* 8:211. <http://dx.doi.org/10.1186/1471-2180-8-211>.
 74. Chauvaux S, Rosso ML, Frangeul L, Lacroix C, Labarre L, Schiavo A, Marceau M, Dillies MA, Foulon J, Coppee JY, Medigue C, Simonet M, Carniel E. 2007. Transcriptome analysis of *Yersinia pestis* in human plasma: an approach for discovering bacterial genes involved in septicemic plague. *Microbiology* 153:3112–3124. <http://dx.doi.org/10.1099/mic.0.2007/006213-0>.
 75. Liu H, Wang H, Qiu J, Wang X, Guo Z, Qiu Y, Zhou D, Han Y, Du Z, Li C, Song Y, Yang R. 2009. Transcriptional profiling of a mice plague model: insights into interaction between *Yersinia pestis* and its host. *J Basic Microbiol* 49:92–99. <http://dx.doi.org/10.1002/jobm.200800027>.
 76. Leskinen K, Varjosalo M, Skurnik M. 2015. Absence of YbeY RNase compromises the growth and enhances the virulence plasmid gene expression of *Yersinia enterocolitica* O:3. *Microbiology* 161:285–299. <http://dx.doi.org/10.1099/mic.0.083097-0>.
 77. Yang F, Ke Y, Tan Y, Bi Y, Shi Q, Yang H, Qiu J, Wang X, Guo Z, Ling H, Yang R, Du Z. 2010. Cell membrane is impaired, accompanied by enhanced type III secretion system expression in *Yersinia pestis* deficient in RovA regulator. *PLoS One* 5:e12840. <http://dx.doi.org/10.1371/journal.pone.0012840>.
 78. Hoe NP, Goguen JD. 1993. Temperature sensing in *Yersinia pestis*: translation of the LcrF activator protein is thermally regulated. *J Bacteriol* 175:7901–7909.
 79. Waldminghaus T, Heidrich N, Brantl S, Narberhaus F. 2007. FourU: a novel type of RNA thermometer in *Salmonella*. *Mol Microbiol* 65:413–424. <http://dx.doi.org/10.1111/j.1365-2958.2007.05794.x>.
 80. Venecia K, Young GM. 2005. Environmental regulation and virulence attributes of the Ysa type III secretion system of *Yersinia enterocolitica* biovar 1B. *Infect Immun* 73:5961–5977. <http://dx.doi.org/10.1128/IAI.73.9.5961-5977.2005>.
 81. Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224. <http://dx.doi.org/10.1093/molbev/msp259>.

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