RNA Regulators: Formidable Modulators of *Yersinia* Virulence

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Summary

A large repertoire of RNA-based regulatory mechanisms, including a plethora of *cis*- and *trans*-acting non-coding RNAs (ncRNAs), sensory RNA elements, regulatory RNA-binding proteins and RNA degrading enzymes have been uncovered lately as key players in the regulation of metabolism, stress responses and virulence of the genus *Yersinia*. Many of them are strictly controlled in response to fluctuating environmental conditions sensed during the course of the infection, and certain riboregulators have already been shown to be crucial for virulence. Some of them are highly conserved among the family of *Enterobacteriaceae,* while others are genus, species or strain-specific and could contribute to the difference in *Yersinia* pathogenicity. Importantly, the analysis of *Yersinia* riboregulators has not only uncovered crucial elements and regulatory mechanisms governing host-pathogen interactions, it also revealed exciting new venues for the design of novel anti-infectives.

Pathogenicity and Virulence Factors in *Yersinia*

The three human pathogenic *Yersinia* species, *Y. enterocolitica, Y. pseudotuberculosis* and *Y. pestis* possess an arsenal of conserved and species-specific virulence factors that promote efficient colonization and persistence of the bacteria in mammals (humans, rodents, pigs/boars, goats, deer, and hares) and insect vectors (fleas). This repertoire enables *Y. enterocolitica* and *Y. pseudotuberculosis* (with 60-70% genomic identity) to cause a range of similar, relatively benign, self-limiting intestinal diseases (yersiniosis). *Y. pestis,* which evolved clonally from *Y. pseudotuberculosis* two to ten thousand years ago (>97% genomic identity), is the causative agent of plague [1, 2]. Currently, the molecular mechanisms that determine the fundamental differences in the pathogenicity between both enteric *Yersinia* species and *Y. pestis* are unclear. Gene gain, loss and rearrangements have been considered to play an important role [3, 4]. Several virulence factors such as the plasminogen activator Pla, the F1 capsular protein and Ymt (*Yersinia* murine toxin) have been found exclusively in *Y. pestis*, whereas the crucial adhesion and invasion factors of the enteric pathogens, *invA* and *yadA*, are inactivated [4]. This could account for their distinct difference in pathogenicity. In addition, there are variations within the regulatory networks controlling expression of virulence-related genes.

Up to now a large repertoire of conserved and species-specific riboregulators, including numerous *cis*- and *trans*-acting non-coding RNAs (ncRNAs), sensory RNA elements (RNA thermometers and riboswitches), regulatory RNA-binding proteins and RNA degrading enzymes, have been identified in pathogenic *Yersiniae,* and many of these riboregulators are part of large regulatory networks. They adjust the expression of colonization factors, toxins, host defense processes and virulence-related physiological and metabolic processes in response to fluctuating environmental conditions encountered during the course of an infection (Figure 1,

Table 1). This review summarizes the current state of knowledge regarding RNA-mediated regulation in *Yersinia*. It describes the characteristics, function and regulation of all classes of *Yersinia* riboregulators with special emphasis on their role in pathogenesis.

The Regulatory Non-coding RNA Repertoire of *Yersinia* which Impacts Virulence

Regulatory ncRNAs are important players of the post-transcriptional regulation of virulence genes. They can interact with ribosomal binding sites or other 'complementary' sites within the coding region of a gene transcript. Such interactions can either modulate translation initiation and efficiency of the targeted mRNA and/or alter the access of RNases leading to an increased or decreased rate of mRNA decay.

Trans-acting RNAs of *Yersinia*

Among the first *trans*-acting small RNAs, which were shown to be important for the control of *Yersinia* virulence, were CsrB and CsrC [5]. Both ncRNAs and the dimeric RNA binding protein CsrA belong to the global carbon storage regulator system (Csr), which has been shown to control various metabolic, physiological and virulence-related genes in response to environmental signals in other bacteria [6-8]. The CsrA protein binds to ANGGA motifs within 5'-untranslated regions (5'-UTRs) (i.e. overlapping the ribosome-binding site) or/and early coding regions of numerous target mRNAs. This leads to altered translation efficiency and transcript stability. CsrB and CsrC form a complex secondary structure with several stem loops with exposed single-stranded GGA motifs that bind and sequester multiple CsrA dimers, thus preventing its activity [5, 8].

The Csr system affects a multitude of physiological and virulence-related activities in *Yersinia*. Initially it was shown that sequestration of CsrA by the Csr RNAs is crucial for the initiation of *Y. pseudotuberculosis* infection. Free CsrA controls expression of the two important colonization factors invasin (InvA) and PsaA through the RovM-RovA regulatory cascade [5, 9, 10] (Figure 1). Deletion of *csrA* also abolishes the motility of *Y. pseudotuberculosis* and *Y. enterocolitica,* as CsrA is required for translation of the flagella master regulator *flhDC* [5, 11]. Flagellar motility is required for efficient adhesion and subsequent invasion into host cells [12]. A microarray analysis further demonstrated that multiple genes of the virulence plasmid associated Ysc type III secretion system are differentially expressed in a *csrA* mutant of *Y. pseudotuberculosis* [13]. Furthermore, the Csr system is implicated in the control of biofilm formation, resistance against antibiotic, osmotic, and heat stress, as well as numerous metabolic pathways [11, 13, 14]. A major feature is that CsrA adjusts the central carbon flow through the pyruvate-acetate node and the tri-carboxylic acid cycle in response to nutrient availability [13].

According to the myriad of influenced physiological activities, it is not surprising that the expression of the Csr system components is tightly regulated by multiple regulatory factors and environmental signals. In contrast to *Escherichia coli* and *Salmonella* Typhimurium, CsrB and CsrC of *Yersinia* are controlled by different two component systems, BarA/UvrY and PhoP/PhoQ (Figure 1). BarA/UvrY senses short chain fatty acids such as formate and acetate in *E. coli* to activate *csrB* [5, 8, 15], whereas the PhoP/PhoQ systems induces expression of the *csrC* gene, depending on the presence of divalent ions (Mg^{2+}, Mn^{2+}, and Ca^{2+}), secreted antimicrobial peptides and acidic pH [16]. PhoP-mediated activation results in transcription of two closely adjacent promoters, leading to two distinct CsrC RNAs. It is unknown whether the two transcripts have different regulatory properties. Furthermore, the cAMP

receptor protein (Crp), a global regulator of ncRNA expression in *Y. pseudotuberculosis [16]*, oppositely influences expression of the small RNAs CsrB and CsrC [17, 18] (Figure 1). This close interplay illustrates that Crp and CsrA are two key players of virulence management. A surprisingly large subset of growth phase responsive ncRNAs, in addition to Csr-type RNAs, was directly as well as indirectly regulated via Crp, which enables virulence and fitness control by ncRNA-based regulatory mechanisms according to the nutritional status of the bacteria [13, 16]. Hence, it is reasonable that the replacement of costly proteinaceous regulatory elements by ncRNAs enhances fitness especially when nutrients are limited.

Several high-throughput RNA sequencing studies combined with sophisticated computational analyses have enabled the identification of more than 500 ncRNA candidates in *Y. pestis* (CO92, KIM6, and biovar Microtus) and *Y. pseudotuberculosis* (YPIII and IP32953) (Supplemental Table S1)[16, 19-24]. The majority of the *Yersinia* ncRNAs (mostly referred to as Ysr based on the initial analysis by Koo *et al*. [19]) were discovered in bacteria grown under different laboratory conditions. Unfortunately, parallel publications have resulted in the use of identical Ysr names for different ncRNAs from *Y. pseudotuberculosis* YPIII and *Y. pestis* CO92 [16, 24], for which we suggest an addition of the first two letters of the first author's surname to avoid confusion (Supplemental Table S1). Most identified ncRNAs are specific for *Yersinia*. This indicates that a large set of these riboregulators is exclusively involved in the fine-tuning of fitness during the different life-styles of this genus.

A thorough comparison of the identified ncRNA repertoires showed a surprisingly low overlap between the five different studies using *Y. pestis* (CO92, KIM6, and biovar Microtus) and *Y. pseudotuberculosis* (YPIII and IP32953) despite the overall high sequence conservation as well as the fact that sequences of almost all identified ncRNAs of these strains could be detected in the genome of IP32953 (Figure 1;

Supplemental Table S1, [16, 25]). Only the highly expressed and most conserved small RNAs were identified in most strains (e.g. MicA, GcvB, GlmZ, Ffs, CyaR, CsrB, CsrC, GlmY, and SsrS). This inconsistency is likely to be the result of different experimental conditions, distinct data analyses and different genome annotations. For instance, several *trans*-acting RNAs in one study (sR028, sR041, sR050, sR066 and sR070) identified in close proximity to adjacent ORFs [23] were reclassified as 5'-UTRs in another study [16]. Possibly, these sRNAs represent premature or processed transcripts of the 5'-UTR (Supplemental Table S1). Moreover, some validated ncRNAs were classified as *trans*-encoded RNAs in *Y. pseudotuberculosis* IP32953, but as antisense RNAs in *Y. pseudotuberculosis* YPIII, as they are encoded antisense to hypothetical proteins, which are not annotated in the IP32953 genome [16]. Furthermore, numerous ncRNAs are differentially expressed and/or contain point mutations, duplications (sibling RNAs), insertions/deletions altering their function and/or stability between and within the species [16, 19, 24, 26, 27]. For instance, the stability of the CsrC RNAs differs significantly between the *Y. pseudotuberculosis* YPIII and IP32953 due to a 20 nt insertion in $csrC_{IP32953}$ [26]. This might partially explain present difficulties faced when attempting to draw conclusions about the presence, type, expression, stability and conservation of a particular ncRNA in the different human pathogenic *Yersinia* species. A more extensive analysis of multiple representative strains of the three human pathogenic *Yersinia* species under an identical experimental set-up and data analysis process is required to elucidate whether a certain identified ncRNA or a variant is specific for the distinct pathogenic properties.

Previous work has shown that the molecular RNA chaperone Hfq, which assists the function of many ncRNAs, is crucial for *Yersinia* pathogenesis [24, 28-31]. This indicates that ncRNAs contribute to *Yersinia* virulence, but which ncRNAs and to

what extent they impact pathogenesis in the different *Yersinia* species is largely unknown. Several of the identified conserved ncRNAs (SgrS/Ysr150, MicF, MicA/Ysr7, GlmY/Ysr147, GlmZ/Ysr148, GcvB/Ysr45, YenS, SraG, FnrS, OmrAB, and Spot42), which are expressed under laboratory growth conditions, are likely to control important metabolic and stress adaptation genes [25, 32, 33]. They are involved in stress adaptation as well as in the outer membrane, cell wall, and iron and carbon metabolism in other *Enterobacteriaceae*, but their role for *Yersinia* virulence has not yet been investigated. So far, only one RNA-seq based approach was performed to profile the expression of *Yersinia* ncRNAs during host colonization. This study demonstrated that a small set of ncRNAs of a *Y. pestis* biovar Microtus strain was at least 4-fold more abundant during murine lung and spleen colonization compared to culture medium [23]. Among these ncRNAs were the *Yersinia*-specific ncRNAs sR039 as well as RyhB1, RyhB2, CyaR/RyeE and RybB, of which only RyhB1 and RyhB2 (Table 1) were further characterized. RyhB1 and RyhB2 abundance is highly increased during iron starvation and dependent on the ferric-uptake regulator Fur [27, 34]. However, neither a *ryhB1* nor a *ryhB2* mutant was attenuated for virulence [27].

Deletion mutants of the conserved and six newly identified ncRNAs of *Y. pestis* biovar Microtus, which were found to be highly abundant in the lung and spleen (sR034, sR035/HmsB, sR055, sR073, sR84, and sR088), were used to infect mice either subcutaneously or intranasally [23]. However, most deletion mutants were not attenuated in virulence. This strongly suggests that these riboregulators fine-tune *Yersinia* fitness and virulence, and are part of robust regulatory networks in which other components can compensate for their function.

In the initial study performed by Koo *et al*. [19], mutants lacking *Y. pseudotuberculosis* ncRNAs Ysr23, Ysr29, and Ysr35 were tested for a virulence phenotype. In

contrast to the Δ*ysr23* strain, infections with the Δ*ysr29* and the Δ*ysr35* strains resulted in a significant difference in survival compared to the wild-type. A deletion of the *ysr35* locus in *Y. pestis* also caused prolonged survival of mice following intranasal infection, indicating that Ysr35 may play a role in pathogenesis. Ysr35 is conserved between the two *Yersinia* species [19]. Its expression has been shown to increase during a thermal upshift supporting its role in virulence [35], but the molecular targets of this ncRNA are still unknown.

More is known about the *Y. pseudotuberculosis*-specific ncRNA Ysr29. A proteomic analysis for Ysr29 target identification revealed multiple proteins contributing to the general stress response as well as environmental and host stresses, including molecular chaperones (GroEL and DnaK), urease (UreC), 30S ribosomal protein S1 (RpsA), glutathione-S-transferase (Gst), peroxidase (AhpC) and the ribosome-recycling factor (Rrf) [19]. Transcript levels of the target genes were not affected by the lack of Ysr29, indicating that this ncRNA controls translation rather than mRNA stability.

A recent genome-wide analysis of ncRNAs expressed by *Y. pestis* further identified an unstable *trans*-acting RNA (Ysr141-SC) encoded upstream of the *yopH* gene of the virulence plasmid pCD1 (Supplemental Table S1). It enhances the synthesis of some type III secretion system (T3SS)-associated effector proteins, e.g. YopJ, YopE, YopK, YpkA and the T3SS/Yop activator LcrF, and reduces production of YopH most likely by altered transcript degradation and translation efficiency [24]. Ysr141-SC regulates YopJ production directly by base-pairing with the 5'-UTR of the *yopJ* transcript. Whether the change of YopJ levels is responsible for the dysregulation of the other T3SS/Yop components, or requires additional control factors is unclear.

Antisense RNAs of *Yersinia*

The genome-wide analysis of the *Y. pseudotuberculosis* YPIII transcriptome further identified 80 putative antisense RNAs of which 19 were encoded on the virulence plasmid pYV [16]. Included are multiple antisense RNAs (asRNAs), which are complementary to the transcripts of the effector YpkA, the V-antigen and several components of the T3SS machinery. This set constitutes a dense community compared to the lower number of antisense RNAs identified on the YPIII chromosome suggesting a fine-tuned control of T3SS/Yop expression. However, the precise molecular function and influence on pathogenesis of these plasmid-encoded asRNAs is yet unknown and much work remains to be done to characterize their influence on *Yersinia* biology.

A recent study demonstrated that *Y. pseudotuberculosis* upregulates the virulence-plasmid copy number during infection, which is indispensable for virulence [36]. Upregulation is caused by an increased expression of the IncFII plasmid replicase RepA, which is repressed under non-infection conditions by the antisense RNA CopA. A marked decrease in CopA levels results in a significant reduction of the ratio of the CopA antisense RNA and the *repA* transcript level in *Y. pseudotuberculosis* during the colonization of the Peyer's patches compared to bacteria grown in culture. How this regulatory tactic is induced during host tissue colonization is still unclear, but the translocon protein YopD was found to influence the copy-number increase [36].

A small number of asRNAs has also been identified in *Y. pestis*. One striking example is a transcript complementary to the plasminogen activator protease gene *pla* on the *Y. pestis* specific pPCP1 plasmid, which includes an ORF for a 48 aa peptide [37]. However, no physiological function has yet been assigned to this anti-*pla* transcript, and no equivalent peptide could be detected. Qu *et al*. identified the asRNAs Yp-sR3, Yp-sR7, Yp-sR8 and Yp-sR16/YsrH on the opposite strand of genes encoding for membrane proteins, the 50S ribosomal protein and FabH an essential

enzyme of fatty acid biosynthesis [21, 38], but their contribution to virulence is not known.

Sensory RNAs in *Yersinia*

Riboswitches and RNA thermometers belong to the class of *cis*-regulatory RNA elements with sensory functions and are typically located within 5'-UTRs. Both types of riboregulators influence downstream gene expression via structural alterations of the sensory RNA segment. While RNA thermometers function as thermo-controlled RNA zippers and typically affect mRNA translation, riboswitches usually alter gene expression on the transcriptional and post-transcriptional levels in response to ligand binding, i.e. binding of metabolites or metal ions.

Riboswitches

The current knowledge of riboswitches in *Yersinia* is rather scarce and is mainly based on a high-resolution whole genome transcriptomics study of *Y. pseudo-tuberculosis* in which numerous mRNAs with long 5'-UTRs (> 200 nt) were discovered [16]. A bioinformatics analysis predicted a few conserved riboswitches (Moco, FMN, cobalamin, yybP-ykoY element), the characterized Mg^{2+}-responsive riboswitch *mgtA [39]*, and 17 additional riboswitch-like elements, indicating that the *Yersinia* riboswitch-repertoire still remains undiscovered. Recently, a powerful unbiased high-throughput approach for global discovery of riboswitches and attenuators (Term-seq) has been described [40]. The application of the Term-seq approach to *Yersinia* grown in different growth media or host environments will not only allow a rapid screening for riboregulators which respond to metabolites of choice, it will also enable the discovery of yet unknown virulence-related riboswitches.

RNA thermometers (RNATs)

Temperature represents a major stimulus for *Yersinia* to adjust metabolism and virulence gene expression, indicating that besides proteineaceous thermosensors such as the virulence regulator RovA [41], RNATs also represent reasonable players in this process. Surprisingly, so far only one RNAT has been identified and characterized in *Y. pseudotuberculosis*, the *lcrF* RNAT located in the intergenic region of the *yscW-lcrF* operon [42]. The transcriptional activator protein LcrF is crucial for the temperature-dependent induction of the virulence plasmid-encoded T3SS machinery and effector proteins. The *lcrF* RNAT consists of a two-stem loop in which the ribosomal binding site (RBS) is hidden in the second hairpin at 25°C. The RBS becomes accessible at 37°C due to structural melting allowing ribosome binding and *lcrF* translation [42, 43]. As RNATs show little to poor sequence and structure conservation, bioinformatic predictions of novel RNATs are limited. However, a very recently performed global analysis of temperature-sensitive RNA structures of more than 1.750 RNAs of *Y. pseudotuberculosis* demonstrated a highly dynamic RNA structurome [44]. This analysis not only showed that reprogramming of the pathogen's virulence program and metabolism in response to temperature is associated with a large restructuring of numerous RNAs, it also led to the identification of 16 novel RNATs. Many of these RNATs are located within 5'-UTRs of transcripts related to virulence, the oxidative stress response, metabolism and heat shock (Figure 2) [44]. In addition to translation, the transcription of many RNAT-controlled genes (e.g. *lcrF*, *ailA*, *cnfY*, *katA*, *sodA*, *sodB* and *sodC*) is also activated upon thermal upshift from moderate to host body temperature [16, 44]. This highlights the importance of a fine-tuned and multilayered control to rapidly adjust the pathogens' demands upon host entry.

Modification of Translation

RNA binding proteins constitute another important class of riboregulators. They affect mRNA translation and turnover in diverse fashion, e.g. they can modulate ncRNA-mRNA interactions and influence the access of the translational and RNA degradation machinery.

Trans-translation by the SsrA-SmpB Tagging and Ribosome Rescue System

The first RNA-based regulatory system shown to influence *Yersinia* virulence was the unique bacterial *trans*-translational control system, composed of the small stable RNA A (SsrA/tmRNA/10Sa/sR022/Yp-sR31) and the small RNA-binding protein B (SmpB) [45]. This system targets premature transcripts that lack stop codons to maintain the translational machinery of the bacteria operational by eliminating ribosomes that are stalled on the damaged/incomplete transcripts via transfer of the polypeptide chain to SsrA [46-48]. Notably, SsrA was among the ncRNAs, which were more abundant during murine lung and spleen colonization of *Y. pestis* biovar Microtus. An equivalent *ssrA* mutant strain was shown to be impaired in replication within macrophages and attenuated for virulence in mice [23]. This can be attributed to the fact that SsrA/SmpB influence (i) expression of the key virulence regulator LcrF, (ii) motility, and (iii) resistance towards oxidative and nitrosative stresses, and low pH [45, 49].

Regulatory RNA-binding Proteins

In addition to the conserved ribosomal and translation proteins, the *Yersinia*-specific translocon protein YopD has a RNA-binding function [50, 51]. YopD (possibly alone or in complex with its secretion chaperone LcrH) was found to bind *yop*-encoding

mRNAs in the 5'-UTRs reducing their translation either by an increase of *yop* mRNA degradation or hindering translation initiation [51]. The precise molecular mechanism is still unknown, but AU-rich regions in close proximity to the ribosome-binding site of the majority of *yop* mRNA seem important but alone are not sufficient for YopD-mediated translational repression [51, 52]. This suggests that other regulatory components contribute this process. In fact, the negative post-transcriptional regulator of the T3SS/Yop system LcrQ (YscM1 and YscM2 in *Y. enterocolitica*) was proposed to assist the YopD-LcrH complex [52]. Moreover, YopD was found to interact with 30S ribosomal particles [53], which suggests perturbation of 30S complex formation and ribosome assembly on the *ysc/yop* mRNAs. A major future challenge will be to unravel the precise mechanism and the dynamic of this multicomponent and multilayered control process that allows the bacteria to carefully balance energy use for virulence factor production and biological fitness/growth.

Changing mRNA Half-lives

Continuous RNA decay is a prerequisite to enable adaptation to rapidly changing environmental conditions such as the ones experienced during the different stages of infection. Several RNA degrading enzymes are known to act cooperatively or independently. They recognize different target sites and degrade their targets with different efficiencies. Several factors such as RNA structure, protection by translating ribosomes and modification of the RNA (e.g. polyadenylation) determine the accessibility and degradation efficiency of these enzymes [54, 55]. Based on the genome sequences all pathogenic *Yersinia* species encode multiple RNases (> 15). Among them are highly conserved endo- and exonucleases, such as polynucleotide phosphorylase (PNPase), RNase E, RNase G, RNase III and RNase Y/YbeY, which cleave internally or from the 3'-/5'-end of RNAs [56] (Table 1). They act on different

pathways to mature rRNAs and tRNAs, process transcripts and intervene in the decay of numerous mRNAs. Together they form a global regulatory network, which is also crucial for the control of virulence gene expression. Despite the fact that these RNases are encoded by human pathogenic *Yersiniae*, not much is known about their implication in RNA-based control mechanisms that influence pathogenesis.

RNase E, a single-strand-specific endonuclease, and the exonuclease PNPase were shown to participate in virulence control. RNase E and PNPase are both required for optimal functioning of the Ysc/Yop T3SS [57-59]. Together with the enzyme enolase and RNA helicase B (RhlB) they form the *Yersinia* degradosome, which suggests that both control T3SS activity in a similar manner [60]. Since the levels of Ysc/Yop transcripts and proteins were increased or remained unchanged in the absence of a functional PNPase or RNase E, they seem to influence transcription/mRNA decay and activity of the T3SS by distinct mechanisms [61]. Moreover, the single-strand specific RNase Y (YbeY) that processes the 3'-ends of the 16S rRNA and is responsible for the late-stage 70S ribosome quality control, also affects many virulence-related features, including acid stress resistance, cell adhesion/invasion properties and T3SS [62]. Among the YbeY-regulated ncRNAs are CsrB and CsrC of the global carbon starvation regulator system, which may explain the pleiotropic virulence-associated phenotypic changes of an *ybeY* mutant.

Concluding Remarks

Recent research led to the discovery of numerous riboregulators and RNA-based regulatory mechanisms evolved by *Yersinia* to control virulence and establish a successful infection. A continuously increasing set of ncRNAs and multiple sensory RNAs that are crucial for virulence have been identified through genome-wide screens. There are certainly more as the vast majority of identified ncRNAs have not

yet been functionally characterized. A major future challenge will be to characterize their molecular targets and control mechanisms, the regulation of their expression and their role for pathogenicity. In addition, numerous intriguing questions and unaddressed issues associated with the *Yersinia* riboregulatory elements remain to be addressed. Some of them are highlighted in the Outstanding Questions.

We eagerly anticipate many exciting new insights into the role of species- and strain-specific riboregulator variants for pathogenesis. A detailed comparison of the identified ncRNAs in different strains of the human pathogenic *Yersinia* species showed that the composition and expression of the ncRNAs vary significantly between the closely related species *Y. pestis* and *Y. pseudotuberculosis* (Supplemental Table S1, [25]), suggesting that specific riboregulators or riboregulator variants could contribute to the pathological differences. However, several intra-species ncRNA variations (e.g. CsrC) or differences in the control of major regulators (e.g. Crp) have also been identified. They have a major influence on the expression of virulence factors [16, 26, 63], making the identification of these potential 'patho-specific' ncRNAs a major endeavour.

Moreover, a rapidly increasing knowledge about the participation of riboregulators in the control of *Yersinia*-host and insect vector interplay is expected, and some of them might represent well-suited drug targets and prone for the development of anti-riboregulation strategies. Promising strategies are the selection of inhibitors that interfere with a central riboregulator such as CsrA [64], the design of tailor-made compounds, or RNAs that interfere with the essential LcrF thermometer, similar to the recently developed riboswitch inhibitors [65, 66].

Taken together, an impressive large repertoire of riboregulators has been recently discovered in the genus *Yersinia,* of which several have already been shown to be formidable modulators of virulence, although, our overall knowledge about their mole-

cular targets, functions and role for pathogenesis is still in its infancy. The ongoing characterization of RNA-based control mechanism will not only open new avenues in our understanding of the control of host-pathogen interactions, it may also pave the way for the development of new anti-riboregulation strategies to combat infections.

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Figure Legends

Figure 1. Regulatory Network of Virulence Factors Controlled by Small ncRNAs in *Yersinia*. Overview of regulatory and environmental factors that control expression of virulence-associated traits in *Yersinia* including host colonization, host-adapted metabolism and immune defense. Transcriptional and post-transcriptional effects and involved ncRNAs (in yellow) are listed.

Figure 2. RNA Thermometers of *Yersinia*. Overview of the identified RNA thermometers of *Y. pseudotuberculosis* controlling virulence, oxidative stress, heat shock and metabolic functions. These all prevent translation of the target mRNAs at 25°C, but not at 37°C, due to incorporation of the ribosome binding site into a thermo-sensitive stem-loop structure. Genes given in red have previously been shown to be temperature-regulated.