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Dual-Function RNA Regulators in Bacteria

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Abstract

The importance of small RNA (sRNA) regulators has been recognized across all domains of life. In bacteria, sRNAs typically control the expression of virulence and stress response genes via antisense base pairing with mRNA targets. Originally dubbed “non-coding RNAs,” a number of bacterial antisense sRNAs have been found to encode functional proteins. Although very few of these dual-function sRNAs have been characterized, they have been found in both gram-negative and gram-positive organisms. Among the few known examples, the functions and mechanisms of regulation by dual-function sRNAs are variable. Some dual-function sRNAs depend on the RNA chaperone Hfq for base pairing-dependent regulation (riboregulation); this feature appears so far exclusive to gram-negative bacterial sRNAs. Other variations can be found in the spatial organization of the coding region with respect to the riboregulation determinants. How the functions of encoded proteins relate to riboregulation is for the most part not understood. However, in one case it appears that there is physiological redundancy between protein and riboregulation functions. This mini-review focuses on the two best-studied bacterial dual-function sRNAs: RNAIII from *Staphylococcus aureus* and SgrS from *Escherichia coli* and includes a discussion of what is known about the structure, function and physiological roles of these sRNAs as well as what questions remain outstanding.

Keywords

Hfq; RNAIII; glucose phosphate stress; SgrS; riboregulation

1. Introduction

In the previous decade, massive research efforts have been aimed at identifying and characterizing novel bacterial small RNA (sRNA) regulators. Genome-wide searches in numerous microorganisms have revealed extensive production of small transcripts, typically in the range of 50 to 350 nt, often encoded in intergenic regions between protein coding genes. While functional characterization of these small transcripts has lagged behind their identification, it is clear that bacteria possess several classes of regulatory sRNAs. One class encompasses members that bind proteins and affect their activities [1–3]; these are not considered further here. Another class of sRNAs contains members that act as antisense RNAs. These sRNAs base pair with and regulate translation and/or stability of mRNA targets [3]. The majority of the sRNAs in this class do not encode protein products and are

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thus commonly known as non-coding sRNAs or riboregulators. In the last several years, however, it has become clear that some antisense sRNAs do encode protein products and are therefore dual-function sRNAs. Only a handful of such dual-function riboregulators have been characterized and these are the focus of this Mini-Review.

Non-coding bacterial riboregulators have been extensively studied in the last decade and numerous unique mechanisms of action have been described. These serve as the basis for current models for riboregulation by dual-function sRNAs. Most well studied non-coding sRNAs are from enteric species, including *Escherichia coli* and *Salmonella enterica*. In these and related organisms, base pairing sRNAs bind to and require the RNA chaperone Hfq for their functions. Hfq binds AU-rich single stranded regions of both sRNAs and mRNA targets [4]. In the absence of Hfq, many sRNAs are unstable and unable to regulate their targets in vivo [3, 5]. In vitro, Hfq has been shown to increase the rate of sRNA-mRNA binding [6, 7] and to remodel RNA secondary structures [8, 9]. These functions of Hfq are presumably important in part because of the short non-contiguous base pairing that characterizes sRNA-mRNA interactions in many gram-negative bacteria. In gram-positive bacteria, there are fewer characterized examples of sRNA-mediated riboregulation. Though many gram-positive species possess Hfq homologs, this protein seems not to play a major role in riboregulation by characterized sRNAs.

Hfq-dependent sRNAs can serve as positive or negative regulators of gene expression (Fig. 1). Positive regulation typically involves sRNA pairing with sequences in an mRNA's 5' untranslated region (UTR) that would otherwise form a translation-inhibitory secondary structure. In these cases, sRNA-mRNA pairing prevents formation of the alternative structure and enhances translation. Negative regulation often involves sRNA-mRNA base pairing interactions that sequester mRNA sequences around the translation initiation site. These interactions inhibit ribosome binding and therefore translation initiation. In many cases, negative regulation of translation is coupled to sRNA-mRNA degradation through the single strand-specific endonuclease RNase E and other associated proteins, collectively known as the degradosome [10–12].

2. RNAIII, a dual-function sRNA in *Staphylococcus aureus*

2.1. Background

S. aureus is an important pathogen that produces numerous cell surface-associated and secreted virulence factors (Reviewed in [13]). Production of these factors is temporally regulated such that surface-associated proteins are produced throughout exponential growth and repressed post-exponentially whereas secreted proteins, including numerous toxins, are only induced post-exponentially. A two-component regulatory system comprised of the AgrAB proteins, which responds to peptide autoinducers of the organism's quorum sensing system, is responsible for the reciprocal regulation of surface-associated and secreted virulence factors. The primary effector of the Agr response is a 514-nt sRNA, RNAIII. RNAIII is produced at the end of exponential phase and is responsible for repressing the synthesis of surface-associated proteins and increasing the synthesis of secreted factors [14]. Nearly all *S. aureus* clinical isolates from acute infections produce RNAIII, highlighting its importance as a central virulence regulator. The RNAIII regulon is large. RNAIII regulates translation and stability of some mRNAs encoding virulence factors via direct base pairing [14–16]. RNAIII also base pairs with and regulates translation of at least one virulence transcription factor [16, 17]; thus, RNAIII indirectly regulates transcription of additional virulence genes. In addition to this riboregulation function, RNAIII encodes a 26-aa secreted peptide, δ -hemolysin. This peptide is not involved in regulating RNAIII target genes.

2.2. Riboregulation by RNAIII

RNAIII has a complex structure composed of fourteen stem-loop (SL) motifs referred to as SL1 through SL14 (5' to 3', respectively) [18]. Three of these SLs contain C-rich sequences in the loops that are likely involved in base pairing with G-rich ribosome binding sites (RBS) [18, 19]. The highly conserved 3' domain of RNAIII is particularly important for its riboregulation function because sequences in this region base pair with several known mRNA targets [16]. SL13 is required for base pairing with sequences around the RBS of *spa* mRNA (which encodes staphylococcal protein A) and repressing its translation [20].

The validated direct targets of RNAIII-mediated regulation are shown in Table 1, along with the RNA regions involved in base pairing interactions. This summary highlights the crucial role of the 3' end of RNAIII in regulation of numerous targets. This region is more conserved in RNAIII from different *S. aureus* isolates than other portions of the sRNA, consistent with a conserved role in riboregulation. At least one target, *hla* mRNA, which encodes α -hemolysin, interacts with sequences at the 5' end of RNAIII [14, 15]. This target is so far the only one that is positively regulated by RNAIII. In contrast, all validated targets that are negatively regulated by RNAIII interact with sequences in the 3' region of RNAIII. Whether differential domains involved in positive *versus* negative regulation of targets is a general rule for riboregulation by RNAIII remains to be determined.

RNAIII was shown to bind Hfq in vivo [20]; however, numerous studies argue against a pervasive role for Hfq in promoting RNAIII-target mRNA interactions [16, 20–22]. One study showed that *hfq* mutations in three different virulent *S. aureus* strains had no significant effects on cell physiology [23]. A more recent study showed a significant stress and virulence phenotype for a different virulent *S. aureus* strain [24]. The authors of this study also that *hfq* expression levels among *S. aureus* isolates were quite variable, which might explain differential roles of Hfq in virulence of distinct isolates. Studies of individual RNAIII-mRNA interactions showed that the stability of RNAIII is not changed in a strain lacking *hfq* [16, 20]. Furthermore, the presence of Hfq does not promote complex formation between RNAIII and *spa* mRNA [20] or RNAIII and SA1000 mRNA [16]. Therefore, unlike riboregulators in gram-negative bacteria, RNAIII does not seem to require Hfq for its stability or interactions with mRNA targets.

The mechanism of direct RNAIII regulation of target mRNAs involves base pairing interactions that affect translation and/or stability of mRNAs. However, it is worth noting that RNAIII controls expression of some genes at multiple levels. For example, the *spa* gene is regulated by RNAIII at the levels of transcription, translation and mRNA stability. *spa* mRNA translation and stability are directly affected through base pairing with RNAIII [20]. Transcription of *spa* is indirectly controlled by RNAIII by virtue of RNAIII control of *rot* expression [16, 20]. Rot is a *spa* activator [25] therefore, RNAIII indirectly represses *spa* transcription by virtue of its direct repression of Rot synthesis. The same mechanisms are likely to control expression of the staphylocoagulase gene *coa* [26].

2.3. Translation of *hld*, encoded by RNAIII

The protein encoded by RNAIII (δ -hemolysin) was identified and characterized long before the riboregulation property of RNAIII was uncovered. The ORF encoding δ -hemolysin is located immediately upstream of the *agr* operon. Interestingly, in addition to the bona fide *hld* coding sequence, three other potential ORFs were identified in the same region; however, translation of these was not detected [27]. δ -hemolysin is a 26-amino-acid peptide secreted by numerous *Staphylococcus* species. This virulence factor targets host cell membranes and causes cell lysis [28]. The main role of δ -hemolysin seems to be its host cell lysis function. Unlike the RNAIII transcript that it is encoded from, δ -hemolysin is thought

to play minimal role in regulatory functions. One study suggested that although δ -hemolysin might slightly upregulate the production of certain exoproteins, it does not affect nearly as many targets as RNAIII [14].

While δ -hemolysin does not seem to have a significant regulatory function, production of this peptide is regulated at multiple levels [27]. First, δ -hemolysin synthesis is controlled at the level transcription of RNAIII, which is stimulated in mid-exponential phase by the response regulator AgrA in response to the accumulation of autoinducing peptides [15]. A second intriguing level of regulation is that of *hld* translation. The 5' and 3' regions RNAIII are involved in intramolecular interactions such that the RBS of *hld* is occluded in a secondary structure [27]. Some as yet uncharacterized event relieves translational repression so that δ -hemolysin begins to accumulate approximately an hour after the synthesis of RNAIII has commenced. Truncations from the 3' end of RNAIII that prevent the formation of the translation-inhibitory structure abolish the delay between RNAIII production and the appearance of δ -hemolysin [27]. The factor(s) mediating RNAIII secondary structure remodeling or processing to allow δ -hemolysin production in vivo are currently unknown. Finally, although it has not been experimentally tested, δ -hemolysin production might be influenced by the riboregulation function of RNAIII. Base pairing interactions between RNAIII and *hla* mRNA involve sequences in the 5' region of RNAIII, overlapping the RBS sequence of *hld* [15]. This pairing would presumably preclude *hld* translation and prevent production of δ -hemolysin.

3. SgrS, a dual-function sRNA in enteric bacteria

3.1. Background

SgrS was first identified in *E. coli* as an sRNA in a global screen for RNA molecules that co-immunoprecipitated with Hfq [5]. At 227 nt, SgrS is longer than typical Hfq-binding sRNAs, which are often between 60 and 100 nt. SgrS is a dual-function sRNA that regulates several mRNA targets through direct base pairing and also encodes a 43-amino-acid peptide, SgrT. SgrS and SgrT are thus far the only documented duo where the riboregulation and protein functions act independently in the same physiological pathway, in this case the glucose-phosphate stress response (Fig. 2).

Glucose-phosphate stress occurs when non-metabolizable sugar phosphates, such as the glucose analogs α -methyl glucoside-6-phosphate or 2-deoxyglucose-6-phosphate accumulate in the cell, resulting in growth inhibition [29–32]. These phosphosugars are generated by transport through the phosphoenolpyruvate phosphotransferase system (PTS). As 2-deoxyglucose (2DG) or α -methyl glucoside (α MG) enter the cell, they are phosphorylated by PTS proteins ManY (EIIC^{Man}) and PtsG (EIICB^{Glc}), respectively. Because these phosphosugars cannot be metabolized by glycolytic enzymes, they accumulate in the cytoplasm. In order to combat the accumulation of these sugar phosphates and resume growth, a stress response is launched that is mediated by a transcription factor, SgrR, which activates transcription of *sgrST* (Fig. 2). The riboregulation function of SgrS serves to repress translation of mRNAs encoding the PTS sugar transporters *ptsG* and *manXYZ* [7, 31–33]. SgrT inhibits sugar uptake through pre-existing transporters by a mechanism that has not yet been elucidated [33]. Together, these two functions reduce accumulation of sugar phosphates and allow cells to continue growing. Without the ability to mount the stress response, *sgrS* mutants are strongly inhibited when grown in the presence of sugar analogs [31–34].

3.2. Riboregulation by SgrS

A study examining the distribution of *sgrR*, *sgrS* and *sgrT* identified orthologs in many enteric species [35]. While the primary sequence of SgrS orthologs was highly divergent,

there was a very short region (~15 nt) of strong conservation near the 3' end of SgrS [35]. These conserved sequences are encompassed in the region involved in base pairing with target mRNAs, *ptsG* and *manXYZ* [7, 31, 32]. Strikingly, structural predictions for these SgrS orthologs indicated that conserved nucleotides reside in a single-stranded region, consistent with the prediction that these residues are involved in intermolecular RNA-RNA interactions [35]. Base pairing between SgrS and *ptsG* mRNA is through a short region of complementarity that involves sequences in the RBS of *ptsG* mRNA [7, 31, 32]. Base pairing leads to translational repression and subsequent coupled degradation of *ptsG* mRNA and SgrS [36]. Regulation of the *manXYZ* polycistronic mRNA by SgrS also occurs by a direct base pairing mechanism. Instead of the typical sRNA-mediated repression involving direct occlusion of the RBS, SgrS base pairs with sequences in the *manX* coding sequence between the 8th and the 11th codons. Base pairing with SgrS leads to degradation of *manXYZ* mRNA [32]. There is minimal overlap between the residues involved in regulating *manXYZ* and *ptsG*. SgrS bases involved in regulating *manXYZ* lie slightly upstream of and are not as strongly conserved as bases in the *ptsG* pairing region. Moreover, two SgrS residues that are indispensable for *ptsG* regulation are not required for regulating *manXYZ* [32]. Computational and experimental evidence indicates that SgrS regulation of *ptsG* is likely to be universally conserved among SgrS orthologs whereas the ability to regulate *manXYZ* is not [32, 35]. Taken together, these results suggest that in organisms that possess SgrS, this sRNA has evolved to regulate a common target, *ptsG* mRNA, as well as other targets that vary among organisms. To date, all confirmed and putative targets appear to interact with the SgrS 3' end [31, 32].

SgrS was identified on the basis of its binding to and co-immunoprecipitation with the RNA chaperone Hfq. *E. coli* *hfq* mutants do not accumulate appreciable levels of SgrS under glucose-phosphate stress conditions, likely because SgrS is very unstable in the absence of Hfq. Not surprisingly, in an *hfq* mutant background, SgrS cannot downregulate *ptsG* mRNA [31]. In vitro studies showed that while Hfq is not strictly required for SgrS-*ptsG* mRNA duplex formation and translational repression [37], Hfq greatly increases the rate of duplex formation [7]. Thus, in contrast to RNAIII, SgrS requires Hfq in vivo for stability and efficient regulation of mRNA targets.

3.3. Translation of *sgrT*, encoded by SgrS

Unlike RNAIII, which has overlapping coding and riboregulation determinants (for regulation of *hla* mRNA), there is a spatial separation of these determinants for SgrS. The *E. coli* *sgrT* coding sequence is located at the 5' end of SgrS (nts 22 through 153) while the sequences involved in base pairing with mRNA targets are located downstream of *sgrT* (from approximately nt 167 through 187). Although these two regions do not overlap, they are only separated by ~15 nt, which encompasses the nt that a ribosome located at the *sgrT* stop codon would occupy. This spacing, as well as the fact that riboregulation of mRNA targets leads to coupled SgrS-mRNA degradation led to the hypothesis that the translation and riboregulation functions of SgrS are mutually exclusive. In other words, a given SgrS molecule could serve as a substrate for translation or as a riboregulator, but not both simultaneously. If this were true, mutations that abrogate translation should lead to enhanced riboregulation activity and vice versa. Preliminary data obtained in the Vanderpool laboratory contradicts this hypothesis and instead suggests a synergy between translation and riboregulation. Mutations that affect ribosome binding or translation initiation actually reduce the ability of SgrS to regulate mRNA targets, even though there is no effect of these mutations on the intrinsic stability of SgrS (Balasubramanian and Vanderpool, unpublished). Further investigation is required to elucidate the relationship between translation and riboregulation for SgrS.

3.4. Function of SgrT protein

Ectopic expression of SgrT from a construct lacking base pairing determinants promotes recovery of *E. coli* cells from glucose-phosphate stress [33]. Expression of base pairing-proficient *sgrS* alleles with mutations in the *sgrT* RBS or start codon likewise rescues cells from stress. Numerous studies have demonstrated that the base pairing function of SgrS acts at the level of translation and stability of target mRNAs and prevents new synthesis of sugar transporters [7, 31–33]. In contrast, SgrT has no effect on target mRNAs, indicating that it must affect recovery from stress via a different mechanism [33]. One study provided evidence that SgrT acts at the level of inhibiting activity of components of the glucose-specific PTS transporter [33]. Studies that are underway will test the hypothesis that SgrT physically interacts with components of this transporter, EIICB^{Glc} or EIIA^{Glc}.

Both SgrS and SgrT are widely conserved among enteric bacteria, however, not all SgrS-containing orthologs encode SgrT, which demonstrates that the riboregulatory function is more conserved than that of the peptide [35]. Expression and regulatory target range varies between the sRNA and peptide as well [34] suggesting that the regulatory roles of SgrS and SgrT have been tailored to fit the physiological needs of individual species.

4. Other dual-function bacterial sRNAs

4.1. SR1 RNA in *Bacillus subtilis*

SR1 is the first dual-function sRNA identified in *Bacillus subtilis*. As a riboregulator, SR1 is responsible for down-regulating arginine catabolism during gluconeogenesis by base pairing with and repressing translation of the *ahrC* mRNA, which encodes a positive regulator of the catabolic *rocABC* and *rocDEF* operons [38]. The SR1-*ahrC* mRNA base pairing is mediated by seven stretches of complementarity within the 3' ends of both RNAs. Base pairing results in structural changes downstream of the *ahrC* RBS that inhibit ribosome binding and thus, translation initiation [39]. Because the binding constant of the SR1-*ahrC* mRNA interaction was lower than for typical sRNA-mRNA interactions, and because both SR1 and *ahrC* bind Hfq, it was initially thought that duplex formation or stability of one or the other partner may be dependent on the RNA chaperone Hfq. However, in vivo and in vitro experiments demonstrated that Hfq does not affect either of these parameters [39], thus the role of Hfq in SR1-mediated regulation remains uncertain.

SR1 was initially described as a non-translated RNA, since translation of a predicted ORF could not be detected from the chromosomal copy of SR1. However, when SR1 was ectopically expressed from a multicopy plasmid, a 39-amino-acid peptide called SR1P was detected. SR1P acts to positively regulate the glycolytic enzyme glyceraldehyde-3-phosphate (GapA), encoded by the *gapA* operon [40]. SR1P was shown to directly bind GapA protein. SR1P also somehow stabilizes *gapA* operon mRNA through an unknown mechanism that does not involve the base pairing function of SR1 sRNA. How the binding of SR1P to GapA relates to the effect on *gapA* mRNA stability is currently unclear, although the SR1 riboregulatory function does not appear to be a factor [40].

An interesting feature of the SR1/SR1P pair is that six out of the seven regions of complementarity between SR1 and *ahrC* mRNA are located within the SR1P coding region. The outlying complementary region, located within the SR1 terminator stem-loop, is required for the initial contact between SR1 and the *ahrC* mRNA, whereas the other regions contribute to pairing efficiency [39, 40]. This arrangement might be important to ensure that SR1 is used to its maximal potential as an RNA molecule by allowing the region of SR1 most critical for pairing initiation to be physically separated from the peptide-coding region.

4.2. PhrS RNA in *Pseudomonas* species

The recently identified bifunctional sRNA PhrS is a regulator of virulence in *P. aeruginosa* [41, 42]. PhrS synthesis is activated by an oxygen-response transcription factor, ANR. One mRNA target of PhrS encodes a primary quorum-sensing regulator, PqsR, which is also a transcriptional activator of a virulence factor in *P. aeruginosa*. PhrS base pairs with a region upstream of the *pqsR* mRNA. Strikingly, instead of base pairing to the RBS of the *pqsR* mRNA itself, PhrS binds to the 5' UTR of an upstream open reading frame (*uof*) whose translation is coupled to *pqsR* translation [41, 42]. The only other sRNA known to carry out a similar mechanism of regulation is RyhB in *E. coli*, which regulates translation of an open reading frame upstream of the *fur* gene [43]. Some of the other putative mRNA targets of PhrS include *cyo* (encoding the terminal cytochrome oxidase), *groEL* (encoding heat-shock proteins), and *oprD* (encoding an outer-membrane porin). The mechanisms of regulation of these targets by PhrS are not yet characterized [42].

PhrS shows a novel mode of Hfq-dependence. Since one of the primary roles of Hfq is to stabilize sRNAs, and since PhrS also co-immunoprecipitated with Hfq, it was hypothesized that Hfq was required for the stability of PhrS [41]. While steady-state levels of PhrS decreased by ~50% in an Δ *hfq* background [41], its stability was found to be independent of Hfq [42]. An intriguing finding was that Hfq indirectly regulates steady-state expression of PhrS by controlling the expression of the transcription factor ANR, which activates the synthesis of PhrS. Nevertheless, Hfq still binds to PhrS and also seems to be required for base pairing of PhrS with its targets [42].

PhrS encodes a 37-amino-acid peptide whose physiological relevance is unknown. Interestingly, the ORF in *phrS* is more highly conserved than *phrS* sequences involved in riboregulation. Although the ORF is conserved among different *Pseudomonas* species, it does not appear to affect riboregulation of *uof-pqrS* [41]. The exact function of the peptide is currently unknown, but it is predicted to act in a pathway different than the PhrS RNA. Notably, the 3' end of PhrS is involved in base pairing and contains a 12 nt highly conserved region (termed *creg*) that is indispensable for PhrS-mediated *uof-pqsR* regulation [41]. This base pairing region is significantly downstream of the ORF. The relationship between riboregulation and translation for PhrS has not yet been investigated.

5. Conclusions

While there is still much that is unknown about dual-function bacterial sRNAs, particularly with regard to the functions of the proteins they encode and the interplay between translation and riboregulation, a few commonalities and differences with non-coding sRNAs can be seen already. Highly conserved regions of the sRNAs are the most likely to be involved in mRNA target regulation. This true for non-coding bacterial sRNAs [44] as well as for dual-function sRNAs like SgrS [35] and RNAIII [16]. A recent study of Hfq-dependent sRNAs suggested that in addition to conservation, a property called accessibility, *i.e.*, the presence of a particular residue in a single-stranded region of the sRNA, predicts whether a residue is involved in intermolecular base pairing interactions with mRNA targets [45]. While such an analysis has not been done for Hfq-independent sRNAs from gram-positive bacteria, it is reasonable to postulate that this is a common sRNA feature.

Involvement of the RNA chaperone Hfq in riboregulation by bacterial sRNAs varies for both dual-function and non-coding sRNAs. Despite the fact that RNAIII and SR1 RNAs bind to Hfq, these and other non-coding sRNAs from gram-positive bacteria typically do not need Hfq for riboregulation. One exception is the sRNA LrhA in *Listeria monocytogenes* that does require Hfq for translational repression of a target mRNA [46]. In contrast, the majority of non-coding sRNAs in gram-negative bacteria like *E. coli* do require Hfq for

sRNA stability and for increasing the rate of sRNA:mRNA interactions. While this is true for the dual-function sRNA SgrS [7], the role of Hfq in PhrS activity is less clear. Interactions with Hfq also differentiate dual-function sRNAs from typical mRNAs. Hfq certainly modulates the stability of some dual-function sRNAs, and may additionally remodel secondary structure and affect translation initiation or elongation. Further, in these sRNAs where the open reading frame and riboregulation determinants are overlapping or very closely spaced, there might be competition for sRNA binding by Hfq and ribosomes. The role of Hfq in modulating production of protein from dual-function sRNAs has not been assessed, and these are interesting issues that will no doubt be the subject of future studies.

Spatial separation of coding versus riboregulatory elements may determine whether the two functions act in tandem or in a mutually exclusive fashion. In dual-function sRNAs such as SgrS and PhrS, these regulatory domains are physically separate, which could potentially enable one RNA molecule to perform two functions simultaneously. Other spatial arrangements, for example in SR1 and RNAIII, utilize the same nucleotide sequences for protein coding and riboregulation, which likely excludes simultaneous use of both functions. Consistent with this idea, for SR1 RNA, riboregulation and SR1P act under distinct environmental conditions, gluconeogenesis and glycolysis, respectively [21, 22, 47].

The physiological roles of proteins encoded by dual-function sRNAs and their relationship to the riboregulatory function is a major outstanding question. δ -hemolysin, a virulence factor, is produced by RNAIII while the riboregulation function is responsible for direct and indirect regulation of other virulence factors. There are hints that translation of *hld* is influenced by the riboregulation function, but there is no clear mechanism to explain how this occurs. The riboregulation function of SgrS and SgrT are clearly connected in that both participate in the same stress response. The SgrS base pairing function works at the level of mRNA target translation and stability, while SgrT appears to act post-translationally on sugar transporter activity. Each can function independently, but recent unpublished data suggest some synergy whereby *sgrT* translation enhances the efficiency of SgrS riboregulation. For SR1/SR1P, the two functions appear to be more independent and mutually exclusive. For PhrS, there is simply not enough information to discern a relationship between riboregulation and protein function. More studies on these and yet-to-be-identified dual-function bacterial sRNAs will shed light on these issues.

Bifunctional sRNAs are emerging as new variations on the theme of regulatory RNAs. Based on the few examples we have so far, it seems we can expect as much diversity among dual-function sRNAs as for non-coding sRNAs and mRNAs. While features such as conservation of the base pairing region may be universal, other properties such as requirement for RNA binding proteins, spatial organization or separation of the two functions and relationships between riboregulatory and protein functions are likely to be highly variable. Identification of more dual-function sRNAs and uncovering their roles in cell physiology will provide useful insight into sRNA evolution and also bridge the gap between the coding and non-coding RNA worlds.

Highlights:

1. Dual-function small RNA (sRNA) regulators are found in both gram-negative and gram-positive species.
2. Highly conserved regions of sRNAs are most likely involved in target mRNA regulation.
3. The RNA chaperone Hfq is typically not needed for riboregulation in gram-positive organisms, but is necessary in gram-negative species.

4. Peptides encoded by sRNA molecules may have physiologically distinct or redundant functions.
5. Spatial organization of coding and regulatory RNA regions may affect function.

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Positive regulation

Negative regulation

Figure 1. Basic mechanisms of regulation by bacterial small RNA regulators

Bacterial sRNAs can positively or negatively regulate expression of target genes. The regulation depends on base pairing with mRNA targets. For a more thorough description of mechanisms of regulation, see Section 1.

Figure 2. The glucose-phosphate stress response of *E. coli*

Non-metabolizable sugar-phosphates including αMG (blue hexagons) and 2DG (purple hexagons) are internalized through the PtsG and ManXYZ PTS transporters, respectively. αMG-6-phosphate and 2DG-6-phosphate accumulate intracellularly. This condition creates an unknown stress signal (orange sun) that is recognized by the transcription factor SgrR, which then activates transcription of *sgrST*. SgrS binds Hfq and base pairs with *ptsG* and *manXYZ* mRNAs to negatively regulate their translation and promote their degradation via RNase E. Production of SgrT inhibits glucose transport via a separate pathway. The current hypothesis is that SgrT interacts with glucose-specific transport machinery (PtsG) and inhibits its activity. The two functions of SgrS promote recovery from stress by reducing the accumulation of growth-inhibitory phosphosugars.

Table 1

Summary of RNAIII-target mRNA interactions.

mRNA Targets ^a	Pairing region RNAIII	Pairing region mRNA	Mechanism of regulation	References
<i>coa</i>	3' end, SL13 (main); also SL7	TIR	RNAIII prevents ribosome binding and translation initiation, and also promotes cleavage by RNase III.	[48]
<i>hla</i>	5' end	5' UTR, upstream of TIR	RNAIII pairing with <i>hla</i> mRNA prevents formation of a translation inhibitory structure and enhances <i>hla</i> translation.	[14, 15]
<i>spa</i>	3' end, SL13	TIR	Direct: RNAIII prevents ribosome binding and translation initiation, and also promotes cleavage by RNase III. Indirect: Transcription of <i>spa</i> repressed by RNAIII, likely through RNAIII repression of <i>rot</i> (see text).	[14, 20, 49]
<i>rot</i>	middle and 3' end, SLs 7, 13, 14	5' UTR upstream of TIR and TIR	Two of three possible pairing interactions between RNAIII and <i>rot</i> mRNA are required for efficient <i>rot</i> translational repression and RNase III cleavage.	[16, 50]
SA1000	3' end, SL13	TIR	RNAIII prevents ribosome binding and translation initiation, and also promotes cleavage by RNase III.	[16]
SA2353	3' end, SL13	TIR	RNAIII prevents ribosome binding and translation initiation, and also promotes cleavage by RNase III.	[16]

^a *coa*, encodes staphylocoagulase; *hla*, encodes α -hemolysin; *spa*, encodes staphylococcal protein A; *rot*, encodes repressor of toxins transcription factor; SA1000, encodes fibrinogen binding protein; SA2353, encodes homolog of secretory antigen precursor.

* TIR, translation initiation region; encompasses ribosome binding site and start codon