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Molecular call and response: the physiology of bacterial small RNAs

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Abstract

The vital role of bacterial small RNAs (sRNAs) in cellular regulation is now well-established. Although many diverse mechanisms by which sRNAs effect changes in gene expression have been thoroughly described, comparatively less is known about their biological roles and effects on cell physiology. Nevertheless, for some sRNAs, insight has been gained into the intricate regulatory interplay that is required to sense external environmental and internal metabolic cues and turn them into physiological outcomes. Here, we review examples of regulation by selected sRNAs, emphasizing signals and regulators required for sRNA expression, sRNA regulatory targets, and the resulting consequences for the cell. We highlight sRNAs involved in regulation of the processes of iron homeostasis (RyhB, PrrF, and FsrA) and carbon metabolism (Spot 42, CyaR, and SgrS).

Keywords

small RNA; RyhB; Spot 42; CyaR; SgrS

1 1. Introduction

How cells are able to sense changes in their environment and respond through alterations in physiology is one of the most fundamental biological questions. In the past decade, small RNAs (sRNAs) have emerged as essential regulators that allow many organisms to properly function and cope with environmental changes and stresses. In bacteria, sRNAs have been discovered to regulate processes as varied as carbon metabolism, iron homeostasis, quorum sensing, RNA polymerase function, virulence, and biofilm formation, a s well as responses to stresses such as oxidation, outer membrane perturbation, and cellular accumulation of sugar-phosphates [1, 2].

Bacterial sRNAs exhibit molecular mechanisms of action that are as diverse as the cellular functions they regulate. One group of sRNAs, riboswitches, consists of RNA sequences located in the 5’ untranslated regions (UTRs) of mRNAs. Riboswitches regulate gene expression by adopting different conformational changes that are mediated by factors such as temperature or small molecule metabolites that bind to the riboswitch. Another class of

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[1]Abbreviations. G6P: glucose-6-phosphate. βMG6P: β-methyl glucoside-6-phosphate. βMG: β-methyl glucoside.

bacterial sRNA acts by binding to protein targets, often sequestering the protein and resulting in decreased activity. A recently defined class of sRNAs called CRISPRs (for clustered regularly interspaced short palindromic repeats) is comprised of repeated sequences alternated with highly variable sequences referred to as spacers that have homology to bacteriophage and plasmid DNA. CRISPRs are thought to provide molecular "immunity" by preventing activity of foreign DNA from "infecting" phages and plasmids. (For extensive reviews on these classes of sRNA regulators and their mechanisms of action, see [2–11]).

The best-characterized class of sRNAs acts through antisense base pairing with target mRNAs. Base pairing sRNAs can be further classified according to their genomic locations relative to those of their mRNA targets: 1) *cis*-encoded sRNAs are located on the strand of DNA opposite their mRNA targets and therefore have extensive complementarity to the target; 2) *trans*-encoded sRNAs are found at genomic locations remote from those of their targets, and their base pairing interactions tend to involve less complementarity. In addition, many *trans*-encoded sRNAs bind to Hfq, a chaperone protein that aids in sRNA stability and/or base pairing with target mRNAs [12]. The antisense base pairing of *cis*-encoded sRNAs with their mRNA counterparts typically leads to negative regulatory effects including RNA degradation, inhibition of translation, or termination of transcription [13]. *Trans*-encoded sRNAs can exert either negative or positive regulatory effects on their mRNA targets (Figure 1). Positive regulation often involves base pairing between the sRNA and mRNA that relieves formation of an intrinsic translation-inhibitory structure in the mRNA's 5' UTR (Figure 1). Several different mechanisms of negative regulation by sRNAs have been reported. In many cases, sRNA-mRNA base pairing interactions occlude the ribosome binding site (RBS) and result in translational repression, which often is coupled to degradation of the sRNA-mRNA complex via the RNase E degradosome pathway [14]. A novel mechanism recently has been described in which the sRNA-mRNA base pairing interaction marks an mRNA for degradation via RNase E without directly affecting translation [15]. Another distinct mechanism of translation inhibition involves an sRNA binding to and occluding CA-rich translational enhancer sequences upstream of the RBS [16]. (For more detailed reviews of basepairing sRNAs and their regulatory mechanisms, refer to [2, 6, 9, 17])

Although the molecular mechanisms which many sRNAs use to regulate their targets have been thoroughly characterized, in general less is known about the environmental and metabolic signals to which they respond and the physiological significance of the cellular changes these regulators effect. Nevertheless, recent work has begun to expand our knowledge of the connections sRNAs have to bacterial physiology and stress responses. In particular, the environmental signals stimulating expression and biological roles of certain *trans*-encoded sRNAs have been uncovered. Typically, expression of these sRNA molecules is regulated by a transcription factor in response to a specific environmental or metabolic signal or stress. Most *trans*-encoded sRNAs can coordinately regulate multiple targets, and some even act as global regulators linking stress responses to numerous other cellular processes. In common with other forms of cellular regulation, the induction and regulation of sRNAs resembles a "molecular call and response" in which environmental or metabolic cues, or "calls," induce expression of the sRNA, eliciting a regulatory "response" that affects gene expression and leads to changes in cellular physiology and/or behavior. In this review, we emphasize what is known about the physiology of sRNAs, including signals, stresses and regulators required for their expression, regulatory targets, and the ensuing consequences for the cell. We focus on examples of Hfq-binding, *trans*-encoded sRNAs that exert regulatory effects on iron homeostasis and carbon metabolism, about which the most regarding physiological inputs and outcomes has been described. These examples are primarily from *Escherichia coli* and *Salmonella*, where the bulk of bacterial sRNA research has been done.

2.1. Small RNAs regulating iron homeostasis

2.1.1. RyhB regulates iron homeostasis in *E. coli*

RyhB, a key regulator of iron homeostasis in *E. coli*, is one of the first sRNAs for which a clear physiological role could be established. RyhB modulates cellular physiology under iron-starvation conditions by regulating a large set of genes that primarily encode iron-containing enzymes. In doing so, RyhB is part of the homeostatic mechanism that maintains a balance between iron uptake and assimilation, which is essential for virtually all microorganisms. As a nutrient, iron is required for the operation of myriad enzymes important in central metabolism, as well as synthesis of DNA and other metabolites. It is crucial for cells to prevent accumulation of excess iron, which promotes formation of highly reactive and damaging oxygen species. While iron generally is abundant in nature, it is poorly bioavailable in aerobic and neutral aqueous environments, and therefore bacterial cells are more likely to encounter iron limitation than iron excess [18, 19]. RyhB redirects cellular iron use under limiting conditions by preventing new synthesis of non-essential iron-containing enzymes, an effect called “iron sparing,” and enhancing the ability of cells to synthesize iron-scavenging siderophores. The importance of RyhB in iron homeostasis is illustrated by the fact that *ryhB* mutant cells have a much longer delay than wild-type cells in exiting lag phase after they have been subcultured into iron-depleted media.

RyhB was first defined in *E. coli* as an sRNA expressed under iron starvation conditions, and is controlled by the ferric uptake regulator (Fur) protein [20]. The Fur protein is the central sensor and regulator of iron homeostasis in many bacterial species, including *E. coli*. Genes encoding siderophore biosynthetic and utilization functions are directly regulated by Fur, which also indirectly controls the prioritization of iron use through repression of *ryhB* transcription. When iron is plentiful, Fur bound to ferrous (Fe^{2+}) iron acts as a transcriptional repressor of a large regulon encoding proteins involved in iron scavenging and assimilation (functions that are not needed when iron is present at high concentrations). When iron is limiting, insufficient Fe^{2+}-Fur results in derepression of genes encoding siderophore biosynthesis and uptake, allowing cells to scavenge iron from the environment. At the same time, *ryhB* repression is relieved and RyhB downregulates targets by direct and indirect mechanisms [21, 22]. mRNAs that are direct targets of RyhB encode non-essential products that require iron for their activities; these mRNAs are rapidly repressed, thereby sparing iron for essential enzymes.

Subsequent to base pairing-mediated r egulation of direct targets, RyhB indirectly represses known Fur-regulated genes. The current rationale for this regulation is that repression of mRNAs encoding iron-containing proteins increases intracellular iron concentrations sufficiently to activate Fur, resulting in Fur target gene repression. Indeed, ectopic expression of RyhB results in a 50% increase in the concentration of free intracellular iron [23]. In addition to iron sparing, RyhB enhances cells’ ability to scavenge limiting iron from the environment by enhancing siderophore biosynthesis. In this way, the concerted actions of Fur and RyhB adjust intracellular pools of available iron.

RyhB has a remarkably broad scope of regulatory effects. A study utilizing microarrays compared gene expression profiles of cells ectopically expressing RyhB to control cells and revealed 56 genes (from 18 operons) as putative targets of RyhB regulation [22]. Validated targets that are regulated by base pairing with RyhB are involved in diverse processes such as siderophore biosynthesis, metabolism (primarily iron-containing enzymes of the TCA cycle), Fe-S cluster biogenesis, iron storage, and respiration. Selected examples of this RyhB-mediated regulation are discussed in more detail below.

RyhB-mediated regulation of metabolism—One of the first RyhB targets to be confirmed was the *sdhCDAB* mRNA, which encodes succinate dehydrogenase, an iron-containing enzyme of the TCA cycle and *sodB*, encoding the iron-dependent superoxide dismutase [20, 24, 25]. RyhB base pairs with sequences in the translation initiation regions of *sdhD* and *sodB* mRNAs, inhibiting translation and promoting degradation of the messages. Similarly, genes encoding several other iron-using TCA cycle enzymes such as fumarate reductase (*frdABCD*), fumarase (*fumA*), and aconitase (*acnA* and *acnB*) are repressed by RyhB [20, 22]. The effects of RyhB on metabolism are more widespread than the TCA cycle. The *nuo* and *fdo* genes, encoding respiratory chain components, are also downregulated by RyhB [22]. In support of the model that RyhB mediates a comprehensive metabolic shift during iron starvation by downregulating pathways with iron-utilizing enzymes, it has been shown that RyhB overproduction prevents cell growth using succinate as a carbon source owing to the RyhB-mediated decrease in succinate dehydrogenase (*sdhCDAB*). Not all iron-containing metabolic genes are RyhB targets, however. Importantly, iron-using enzymes such as ribonucleotide reductase (for DNA precursor synthesis) and dihydroxyacid dehydratase (for amino acid biosynthesis) are not subject to downregulation by RyhB under iron-starvation conditions.

RyhB-mediated regulation of siderophore biosynthesis—A recent study uncovered a crucial role for RyhB in enterobactin siderophore biosynthesis [26]. During iron starvation, Fur derepression leads to transcription of the *entCEBAH* genes needed for the biosynthesis of enterobactin [27]. *ryhB* mutants produce significantly less enterobactin than wild-type cells under conditions of iron starvation. This is due both to a defect in *entCEBAH* expression in *ryhB* mutants (caused by an as yet unknown mechanism) as well as reduced availability of L-serine, an intermediate required for the final steps of enterobactin biosynthesis [26]. RyhB affects availability of L-serine by repressing translation of *cysE*, which encodes the first enzyme in cysteine biosynthesis. RyhB inhibits translation initiation and stimulates *cysE* mRNA degradation by base pairing with sequences near the RBS. Salvail, *et al.*, proposed that when iron is scarce, negative regulation of *cysE* translation by RyhB reduces the flow of L-serine to cysteine biosynthesis in order to spare it for enterobactin biosynthesis [26]. Consistent with this idea, enterobactin production was restored to the *ryhB* mutant by mutation of *cysE* or supplementation with exogenous serine [26].

While most RyhB mRNA targets are repressed, at least one target, *shiA* mRNA, is stabilized and translationally activated as a result of base pairing with RyhB. In the absence of RyhB, the 5’ UTR of *shiA* mRNA forms a secondary structure that sequesters the ribosome binding site and therefore represses translation [28]. When RyhB is produced under iron starvation conditions, it base pairs with a portion of the *shiA* mRNA 5’ UTR and prevents formation of the translation-inhibitory structure. This results in increased translation and enhanced stability of *shiA* mRNA. The *shiA* gene encodes a permease for shikimate, a precursor in the enterobactin biosynthesis pathway. RyhB-enhanced translation of *shiA* in iron-depleted environments would allow cells to acquire a precursor that facilitates increased siderophore production, providing shikimate is present. Thus, RyhB affects enterobactin siderophore synthesis through regulation of other connected metabolic pathways.

2.1.2. Regulation of iron homeostasis by sRNAs in organisms other than *E. coli*

BLAST searches for sRNAs with similarity to *E. coli* RyhB identified putative RyhB orthologs in other enterobacterial species [20] but not in organisms beyond this family. However, orthologs of genes regulated by RyhB in *E. coli* have been shown to respond to similarly to iron and Fur in *Pseudomonas aeruginosa* [29], leading to the hypothesis that RyhB-like mechanisms of regulation might exist more broadly. A bioinformatic search

identified two sRNAs named PrrF1 and PrrF2 (for *Pseudomonas* regulatory RNA involving iron) encoded in tandem in an intergenic region [30]. PrrF1 and PrrF2 are each ~110 nt in length and are >95% identical to one another, but share no similarity with *E. coli* RyhB. Despite the lack of sequence identity, regulation of PrrF1 and PrrF2 by Fe^{2+}-Fur and the iron sparing function are analogous to RyhB physiology [30]. Their expression is induced in iron-limiting conditions, and target transcripts include *sdh*, *sodB*, and bacterioferritin mRNAs [30]. Little else is known about their physiological roles in the cell except for the fact that PrrF1 and PrrF2 appear to be functionally redundant. Deletion of one or the other copy has little effect on gene regulation or cell growth, whereas deletion of both copies relieves repression of mRNA targets [30]. A recent study [31] suggested a unique metabolic connection for the Prr RNAs. Read-through of the PrrF1 terminator generates an ~325 nt species that terminates at the PrrF2 terminator. This longer RNA has been named PrrH and it appears to have targets that are not shared with either PrrF1 or PrrF2.

Bacillus subtilis encodes a Fur-regulated sRNA called FsrA that also mediates an iron-sparing response via downregulation of mRNAs encoding iron-using enzymes, *e.g., sdh* [32]. As with PrrF1 and 2, other physiological relevance has yet to be ascertained. Unlike RyhB and the PrrF sRNAs, the *B. subtilis* FsrA sRNA does not require Hfq for its activity. Instead, three small basic proteins, FbpA, FbpB and FbpC participate in regulation of mRNA targets. The current hypothesis is that these three small proteins act as chaperones for FsrA and aid in regulation of different subsets of mRNA targets.

2.2. Small RNAs regulating carbon metabolism

2.2.1. Spot 42 globally regulates alternative carbon source utilization

One of the best-characterized sRNAs in regard to physiological signals and regulatory outcomes is Spot 42. Identified decades ago in *E. coli*, regulation of Spot 42 expression and some of its phenotypic effects have long been recognized, but it was relatively recently that one of its primary physiological functions was determined [33]. Although *spf* (named for spot forty-two) mutants do not exhibit significant phenotypic changes [34, 35], overexpression of Spot 42 leads to multiple defects in growth, including smaller colony size, a lag in growth upon transfer from minimal to to rich media, and impaired growth with nutrient sources such as succinate [36]. In addition, it is well-established that expression of Spot 42 is repressed by the cAMP-CRP (cAMP receptor protein) complex. When the cAMP-CRP complex is active, as in cells grown with cAMP or in the absence of glucose, levels of Spot 42 transcript were found to decrease significantly [35, 37], implying a potential physiological role for Spot 42 in carbon metabolism.

However, it was not until 2002 that a regulatory target of Spot 42 was identified. Spot 42 inhibits translation of *galK*, one member of the *gal* galactose utilization operon, while the expression of two other operon members, *galE* and *galT*, is relatively unaffected [33]. Spot 42 was shown to prevent ribosome binding through specific antisense base pairing with the ribosome binding site of *galK*, while still allowing the upstream *galE* and *galT* mRNA sequences to be translated [33]. The regulatory connection between Spot 42 and cAMP-CRP is illustrated by the fact that cells lacking CRP exhibit an increase in this discoordinate expression of the *gal* transcript [33, 38–40].

Møller et al. proposed that differential regulation of the *gal* operon allows optimal use of available carbon sources. Under conditions where galactose is the available carbon source, all three enzymes are needed for its metabolism [33]. During growth on other carbon sources such as glucose, cells still require GalT and GalE to make a small amount of certain galactose derivatives for use in biosynthesis of lipopolysaccharides [38], but GalK is not needed for this process. Thus, when the preferred carbon source glucose is absent, Spot 42 is

repressed and production of all the enzymes needed for galactose metabolism can take place. Conversely, when glucose is present, Spot 42 decreases production of GalK, while GalT and GalE can still function in lipopolysaccharide synthesis [33]. In sum, an environmental condition (the presence of glucose) and signal (low level of cAMP) result in activation of an sRNA (relief of cAMP-CRP repression of Spot 42), which leads to regulatory changes with observable effects on cell physiology (down-regulation of the unneeded portion of the galactose utilization pathway).

The known regulatory roles of Spot 42 recently have expanded beyond galactose utilization to a broad assortment of metabolic effects ranging from redox balance to transport and metabolism of a variety of carbon sources [41]. During growth in glucose, Spot 42 was found through microarray analysis to decrease levels of transcripts such as *srlA* (involved in sorbitol transport), *fucI* (required for fucose catabolism) and *sthA* (necessary for the oxidation of NADPH via NAD^+) [41]. Regulation of these genes was confirmed with *lacZ* translational fusions, and Spot 42 was demonstrated to base pair specifically with several of these mRNA targets. *spf* overexpression also resulted in decreased growth on relevant nutrient sources, including sorbitol and fucose. While wild-type cells showed little to no expression of SrlA and FucI proteins when grown with glucose (i.e., when Spot 42 is typically expressed), deletion of *spf* led to increased (or “leaky”) expression [41]. This leaky expression phenotype, along with the fact that many of the new Spot 42 targets are known to be activated by cAMP-CRP in the absence of glucose, led Beisel a nd Storz to suggest that Spot 42 and cAMP-CRP comprise a novel “feedforward loop” in which cAMP-CRP represses Spot 42, while both cAMP-CRP and Spot 42 regulate (positively and negatively, respectively) the same target genes [41]. In other words, targets of Spot 42 repression also are positively controlled at the level of transcription by cAMP-CRP. As a result, in conditions of high glucose (and low cAMP), transcription of target genes is reduced and at the same time, increased Spot 42 reduces target transcript levels even further. Though not yet definitively known, the authors proposed that the physiological purpose of this regulatory loop could be (at least in part) to diminish leaky expression of genes that are not necessary for growth in glucose, thus increasing the efficiency of cellular metabolism and energy use [41].

2.2.2. CyaR: a recent addition to global sRNA regulators

Among characterized bacterial sRNAs, one of the widest regulatory ranges belongs to CyaR, which has been demonstrated in *E. coli* and *Salmonella enterica* serovar Typhimurium to regulate a hodgepodge of targets involved in NAD synthesis, outer membrane stress, and quorum sensing [42–44]. Like Spot 42, expression of CyaR is regulated by cAMP-CRP. Unlike Spot 42, however, CyaR is *activated* by cAMP-CRP, and its confirmed targets have no obvious connections to carbon metabolism. Under conditions of low glucose (high cAMP), CyaR activation leads to decreased mRNA levels of genes encoding an outer membrane protein (*ompX*), an NAD synthetase (*nadE*), and a quorum sensing autoinducer synthase (*luxS*) [42–44]. *E. coli* CyaR has been demonstrated to work by directly base pairing to the ribosome binding sites of these target transcripts, and, in the case of *luxS*, it leads to decreased production of autoinducer-2[42]. Autoinducer-2 has important roles in the expression of numerous genes, such as those involved in motility and biofilm formation [45–47].

Despite (or perhaps because of) its wide array of cellular effects, the biological significance of CyaR so far remains elusive. The potential regulatory linkage via CyaR of carbon metabolism (CRP) to motility and biofilm formation (LuxS) is reminiscent of the protein-binding sRNAs CsrB and CsrC, part of a regulatory network connecting carbon storage, motility and biofilms. (Reviewed in [4, 48].) Given that CyaR decreases levels of OmpX (an outer membrane protein with a potential but as-yet-unconfirmed role in adherence) and

LuxS (which incites biofilm formation) in low glucose conditions, De Lay and Gottesman speculated that CyaR may, under nutrient-poor conditions, promote cells' search for improved nutritional sources [42].

2.2.3. SgrS regulates the response to sugar-phosphate stress

Bacteria experience stress when sugar-phosphates such as glucose-6-phosphate (G6P) or non-metabolizable analogs such as α-methyl glucoside-6-phosphate (αMG6P) accumulate in the cell and block downstream steps in glycolysis. When left unimpeded, sugar-phosphate accumulation can prevent cell growth (as in the case of G6P and αMG6P) [49] or lead to death [50, 51], although the cause of stress and mechanisms underlying growth inhibition and cell death are not currently known. One strategy employed by some enteric bacteria, including *E. coli*, for dealing with glucose-phosphate stress is the use of a dedicated regulatory system comprised of the transcriptional regulator SgrR and the sRNA SgrS (named for sugar transport-related sRNA), both of which are essential for mediating the response to and recovery from glucose-phosphate stress (Figure 2). The growth of *sgrR* and *sgrS* mutants is severely inhibited during glucose-phosphate stress, while wild-type *E. coli* is able to recover [52, 53].

Through an unknown signal, accumulation of G6P or αMG6P activates SgrR [52, 54–57] and promotes transcription of the *sgrS* gene (Figure 2). In *E. coli*, both glucose and α-methyl glucoside (αMG) can be transported into the cell and concomitantly phosphorylated by the *ptsG*-encoded $EIICB^{Glc}$ transporter, as well as by certain other phosphoenolpyruvate phosphotransferase system (PTS) transporters. SgrS negatively regulates *ptsG* mRNA at a post-transcriptional level, preventing new synthesis of the glucose transporter $EIICB^{Glc}$ and thus inhibiting further accumulation of G6P or αMG6P [52, 53, 58]. SgrS acts through specific base pairing with *ptsG* mRNA, resulting in inhibition of translation and subsequent degradation of the *ptsG* transcript via the RNase E degradosome [53, 58, 59]. SgrS has a novel, distinct second role: it encodes a small protein, SgrT, which inhibits $EIICB^{Glc}$ activity (and thus glucose-phosphate transport) through an as-yet unknown mechanism [60].

Recent work has demonstrated that SgrS has additional regulatory targets, including at least one other PTS transporter: as with *ptsG*, SgrS down-regulates levels of the *manXYZ*-encoded $EIIABCD^{Man}$ transporter, which is capable of bringing mannose and (to a lesser extent) glucose into the cell, by inhibiting translation of the *manXYZ* transcript [61] (Figure 2). Like SgrS, SgrR has multiple regulatory targets: it positively regulates transcription of at least two other genes [57, 62], *setA* and *yfdZ* (recently renamed *alaC* for a role in alanine synthesis [63]), which encode, respectively, a sugar efflux pump and a glutamic-pyruvic transaminase. Although not the major efflux transporter of glucose during stress, *setA* is required for full recovery from stress, and SetA is thought to pump stress-related metabolites out of the cell [62]. The role of AlaC in glucose-phosphate stress is unknown, but its glutamic-pyruvic transaminase activity, which is capable of generating pyruvate [63], may alter metabolism downstream of the glycolytic block [57].

Physiology: the unknown of the glucose-phosphate stress response— Although the molecular mechanisms of the glucose-phosphate response are well characterized, the biological role of the stress response, the inducing signal, and the cause of glucose-phosphate stress all have yet to be established. The molecular effectors of the stress response (SgrR, SgrS, and SgrT) are conserved not just in *E. coli* but among many members of Enterobacteriaceae [54, 64]. While some enteric bacteria can prevent accumulation of sugar-phosphates through SgrR and SgrST, others, such as *Klebsiella* species, may be able to mitigate the effects of glucose-phosphate stress by actually metabolizing αMG [65]. Thus, while the biological significance is not known, the presence of varied and specific sugar-phosphate stress responses suggest the stress is relevant in natural conditions.

The SgrR-SgrST stress response appears to be active against other substrates, such as the glucose analog 2-deoxyglucose, and stress induction is affected by the presence or absence of transporters with different sugar specificities [61], (Y. Sun and C. Vanderpool, unpublished data). While the PtsG transporter has a higher affinity for αMG than 2-deoxyglucose, ManXYZ is a better transporter of 2-deoxyglucose than αMG [66–70]. Consistent with this, *sgrS* expression is much more highly induced in a Δ*manXYZ* mutant than a Δ*ptsG* mutant during growth in the presence of αMG, while the opposite is true when 2-deoxyglucose is the stressor [61]. (Δ*manXYZ* Δ*ptsG* double mutants are largely resistant to stress regardless of the sugar substrate [61].)

While the signal that leads to activation of SgrR and production of SgrS during glucose-phosphate stress has not been identified, some circumstantial evidence points to it being a small molecule, and likely not glucose-phosphate itself. First, the family of transcriptional regulators to which SgrR belongs possesses an N-terminal DNA-binding domain and a C-terminal solute-binding domain, the latter of which may be important for SgrR to bind the stress signal [52]. Second, ectopic expression of SgrR can only activate *sgrS* transcription under sugar-phosphate stress conditions, which implies that the activity of SgrR protein must be regulated by the unknown signal [52]. Third, the fact that SgrR-regulated AlaC is capable of generating pyruvate [63], the final product of glycolysis, suggests a potential link of glycolytic intermediates to activation of the glucose-phosphate response regulator.

Sugar-phosphate stress has long been known [49–51, 71, 72], but the physiological cause is uncertain and could be due to either toxicity of high sugar-phosphate levels [53, 73] or depletion of glycolytic or other downstream metabolites [74]. Some evidence suggests that depletion of downstream metabolites, rather than toxicity of high G6P levels *per se*, is the more likely cause of stress [53, 59, 74]. Adding glycolytic intermediates rescues phosphoglucose isomerase *pgi* mutants (which accumulate G6P) from glucose-phosphate stress. For example, stress can be alleviated by the addition of glycolytic compounds such as fructose-6-phosphate and pyruvate downstream of the metabolic block [75]. Moreover, stress also is induced by accumulation of fructose-6-phosphate (*pfk* mutants) and fructose-1,6 bisphosphate (*fda* mutants) and adding relevant compounds downstream of the particular metabolic block again rescues cells from stress [73, 75]. Together, these observations suggest that stress is not a result of accumulation of a particular sugar-phosphate; rather, levels of phosphoenolpyruvate (PEP), the penultimate intermediate in glycolysis, could be the key to the stress response. PEP connects glycolysis to sugar-phosphate transport; it is the first phosphate donor in the relay that activates PTS transporters such as SgrS targets PtsG and ManXYZ. During transport of glucose or αMG, a block in the glycolytic pathway could lead to a decrease in the amount of available PEP, which itself could be responsible for the growth inhibition observed in *pgi* and *sgrS* mutants during glucose-phosphate stress [74].

Other sugar-phosphate stresses are largely uncharacterized, with the exception of stress induced upon UDP-galactose accumulation in a *galE* mutant strain exposed to galactose [76, 77]. In this system, the accumulation of phosphosugars (UDP-galactose) is apparently not the cause of cellular stress but rather, the concomitant depletion of UTP and subsequently CTP (derived from UTP). Growth inhibition of *galE* mutant strains growing with galactose is therefore largely reversed by supplementation with pyrimidines [76].

Recently regulatory clues have also expanded the connections of glucose-phosphate stress to other aspects of cell physiology. During growth with αMG to induce stress, mutating *crp* leads to a decrease in *sgrS* induction compared to wild-type, while mutating *kdgR*, which encodes a transcriptional regulator of carbon transport and catabolism genes [78, 79], results in a slight increase in *sgrS* expression [62]. Both appear to affect *sgrS* transcription through

regulation of SgrR activity, as ectopic expression of active SgrR (in the *crp* and *kdgR* mutants) abrogates these *sgrS* expression differences [62]. Consistent with this, a *crp* mutant exhibits decreased growth compared to wild-type when αMG is used to induce stress on certain M63 minimal media, but the *crp* growth defect is not as severe as that of an *sgrR* mutant (G. Richards and C. Vanderpool, unpublished data). It is perhaps no surprise that CRP-cAMP, active under conditions of low glucose, is a potential positive regulator of the glucose-phosphate stress response: with glycolysis blocked during glucose-phosphate stress, the cell likely senses it is starved for glucose. Intriguingly, KdgR represses expression of *eda*, which encodes an aldolase whose products are triose-3-phosphate and pyruvate [78]. Since pyruvate was shown to decrease *ptsG* mRNA degradation (and perhaps ameliorate stress) in a *pgi* mutant [75], the *kdgR* mutant may exhibit increased *sgrS* expression due to altered cellular levels of pyruvate.

3. Conclusion

Traditionally, much of the research on bacterial sRNAs has focused on the diverse molecular mechanisms by which they bring about regulatory changes in the cell. Recent research has uncovered important physiological ramifications of regulation by some sRNAs in terms of the environmental and metabolic “calls” that induce sRNA expression, and the “response” of resulting changes in cell metabolism and/or behavior. For every sRNA with an established biological role, the roles of countless others have not yet been determined. As more sRNA regulatory mechanisms continue to be described, it will remain equally important to place this regulation in a biological context, particularly given the complex and ever-expanding array of metabolic and behavioral processes targeted by sRNA regulation.

Highlights

- Bacterial sRNAs are important regulators with well-described molecular mechanisms.
- Relatively less is known about the effects of sRNAs on cell physiology and behavior.
- We review environmental and metabolic cues and regulators needed for sRNA activation.
- We also emphasize sRNA targets and what is known about physiological effects.
- We focus on selected sRNAs involved in iron homeostasis and carbon metabolism.

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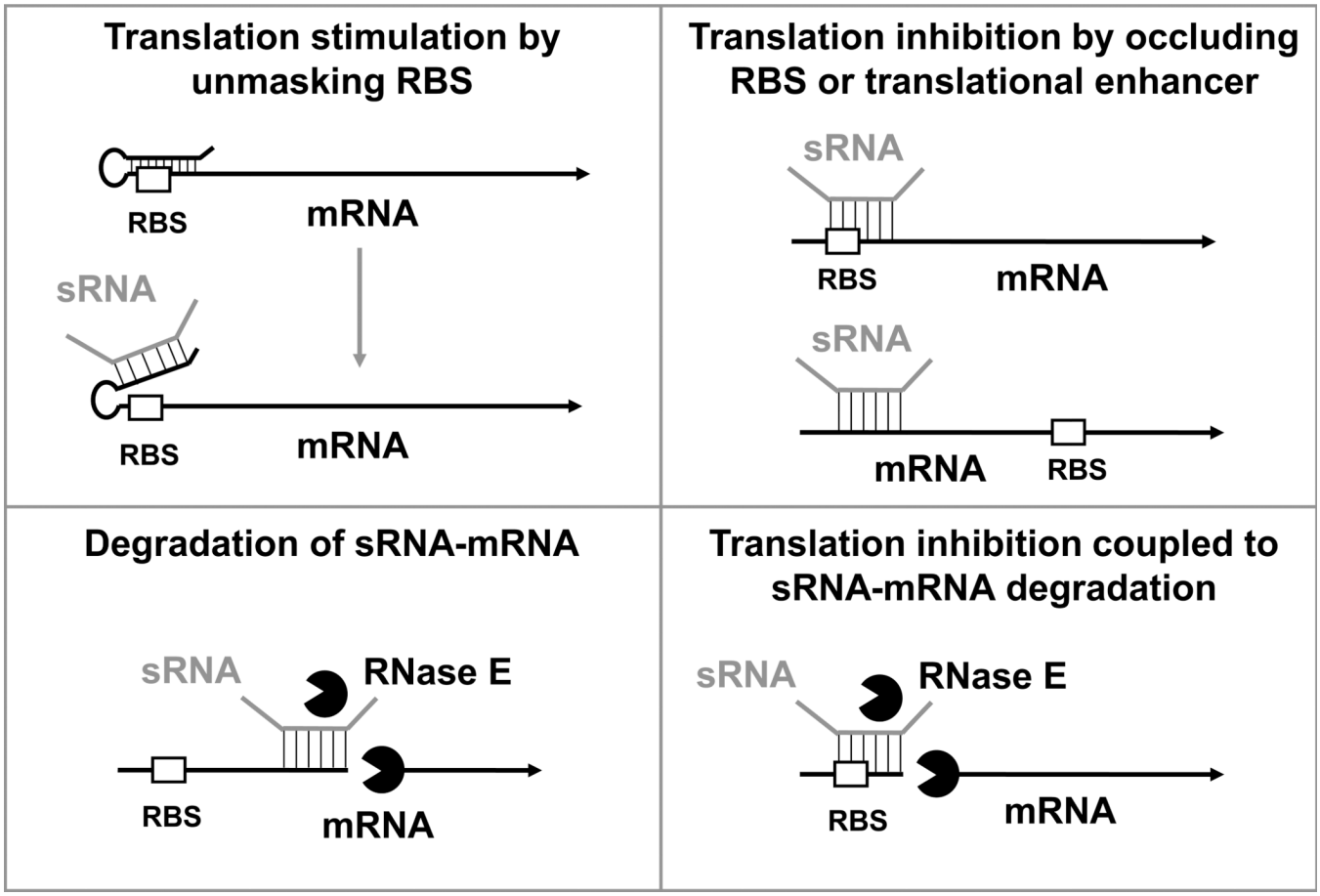

Figure 1.

Selected mechanisms of regulation by *trans*-encoded sRNAs. Small RNAs (sRNAs) are designated by gray lines, and their messenger RNA (mRNA) targets are denoted by black arrows. White boxes represent ribosome binding sites (RBSs) of mRNAs. Series of small, thin lines signify complementary base pairing between an sRNA and its target mRNA. mRNA degradation by RNase E degradosome (black, wedged circles) is represented by broken black arrows. Gray arrow denotes transition before and after sRNA base pairing. See Section 1 for details.

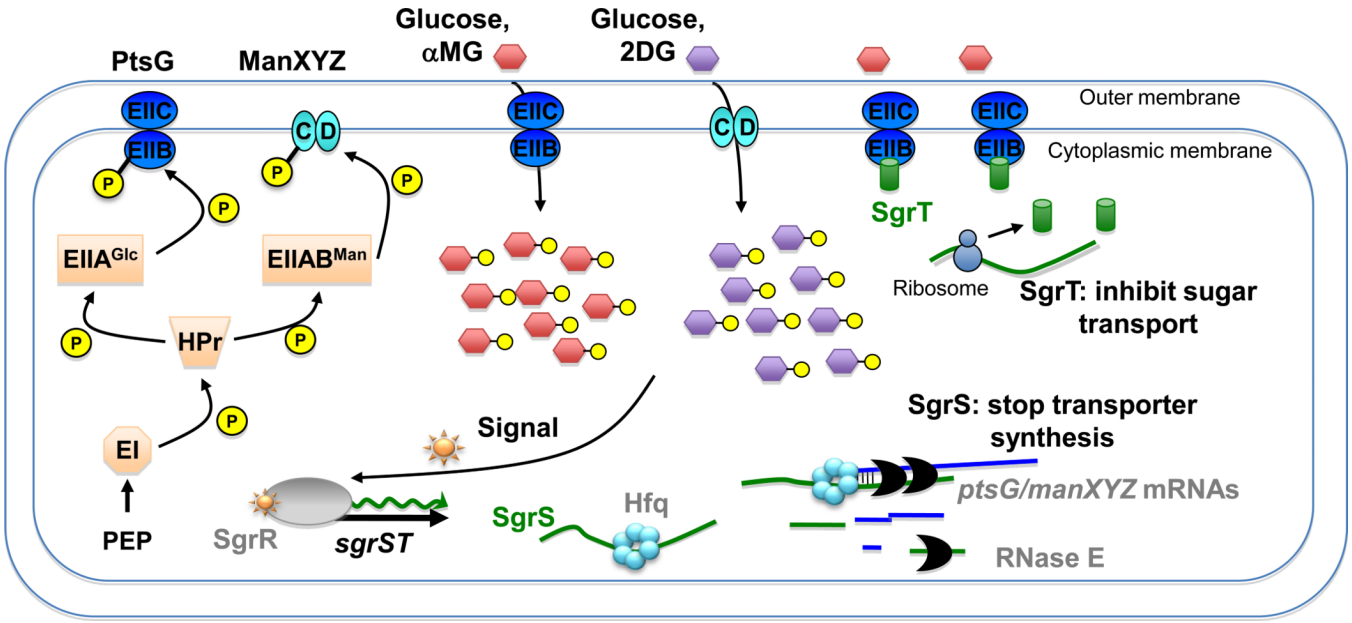

Figure 2.

Regulation and physiology of the response to glucose-phosphate stress. The PTS phosphorelay cascade (orange) leads to activation/phosphorylation (yellow circles) of both the PtsG (dark blue) and ManXYZ (light blue) sugar transporters. Glucose-phosphate stress occurs when glucose or its analogs αMG (red hexagons) and 2DG (purple hexagons) are phosphorylated upon entry through the transporters and accumulate in the cell, inhibiting growth. An unknown signal (gold sun shape) activates the transcriptional regulator SgrR (gray), which in turn activates transcription of the regulatory sRNA SgrS (green line). Associated with the sRNA chaperone Hfq (light blue hexamer), SgrS base pairs with both *ptsG* and *manXYZ* mRNAs (blue lines), blocking translation and resulting in subsequent degradation by the RNase E degradosome (black, wedged circles). In addition, SgrS encodes a small peptide, SgrT (green cylinder), which inhibits the sugar transport activity of PtsG through an undefined mechanism. See text for details.

Table 1

Regulation and physiological effects of sRNAs covered in this review.

Cue/signal	Regulator[a]	sRNA[b]	Target genes	Effect(s) on cell physiology/behavior
Low Fe	Fur (−)	RyhB[c] (+/−)	*sdhCDAB*, *sodB*, *nuo*, *fdo*, *entCEBAH*, *cysE*, *shiA*, other TCA genes, *et al.*	Fe sparing due to decrease in nonessential Fe-using enzymes; Fe acquisition due to increased siderophore (enterobactin) synthesis; decrease activities of TCA cycle and respiratory chain
Low Fe	Fur (−)	PrrF1, PrrF2[d] (−)	*sdhCDAB*, *sodB*	Fe sparing due to decrease in nonessential Fe-using enzymes
Low Fe	Fur (−)	FsrA[e] (−)	*sdhCDAB*	Fe sparing due to decrease in nonessential Fe-using enzymes
High glucose/low cAMP	CRP (−)	Spot 42[c] (−)	*galK*, *srlA*, *fucI*, *sthA*, *et al.*	Decrease galactose utilization while maintaining levels of Gal enzymes needed for LPS synthesis; decrease alternative carbon source metabolism
Low glucose/high cAMP	CRP (+)	CyaR[c,f] (−)	*ompX*, *nadE*, *luxS*, *et al.*	Decrease OMP synthesis and quorum sensing; increase biofilm formation and motility
phosphosugars/unknown	SgrR (+)	SgrS[c] (−)	*ptsG*, *manXYZ*	Prevent sugar uptake, reduce accumulated sugar-phosphates

[a] Symbol in parentheses denotes regulatory effect on sRNA transcription. (+), positive regulation; (−), negative regulation. See text for details.

[b] Symbol in parentheses denotes sRNA regulatory effect(s) on mRNA targets. (+), positive regulation; (−), negative regulation; (+/−), positive or negative regulation, depending on target. See text for details.

[c] Based on studies in *Escherichia coli*.

[d] Based on studies in *Pseudomonas aeruginosa*.

[e] Based on studies in *Bacillus subtilis*.

[f] Based on studies in *Salmonella enterica* serovar Typhimurium.