



## Mini review

## Bacterial sRNAs: Regulation in stress

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## ABSTRACT

Bacteria are often exposed to a hostile environment and have developed a plethora of cellular processes in order to survive. A burgeoning list of small non-coding RNAs (sRNAs) has been identified and reported to orchestrate crucial stress responses in bacteria. Among them, *cis*-encoded sRNA, *trans*-encoded sRNA, and 5'-untranslated regions (UTRs) of the protein coding sequence are influential in the bacterial response to environmental cues, such as fluctuation of temperature and pH as well as other stress conditions. This review summarizes the role of bacterial sRNAs in modulating selected stress conditions and highlights the alliance between stress response and clustered regularly interspaced short palindromic repeats (CRISPR) in bacterial defense.

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## Introduction

Bacteria encounter a wide range of stresses in their constantly changing environments. Variations in temperature, pH, solute concentrations, nutrients, and oxygen level can exert environmental stress on their growth. In order to adapt and survive in an often-hostile atmosphere, bacteria have developed ways to sense changes and orchestrate a cascade of alterations in gene expression and protein activity.

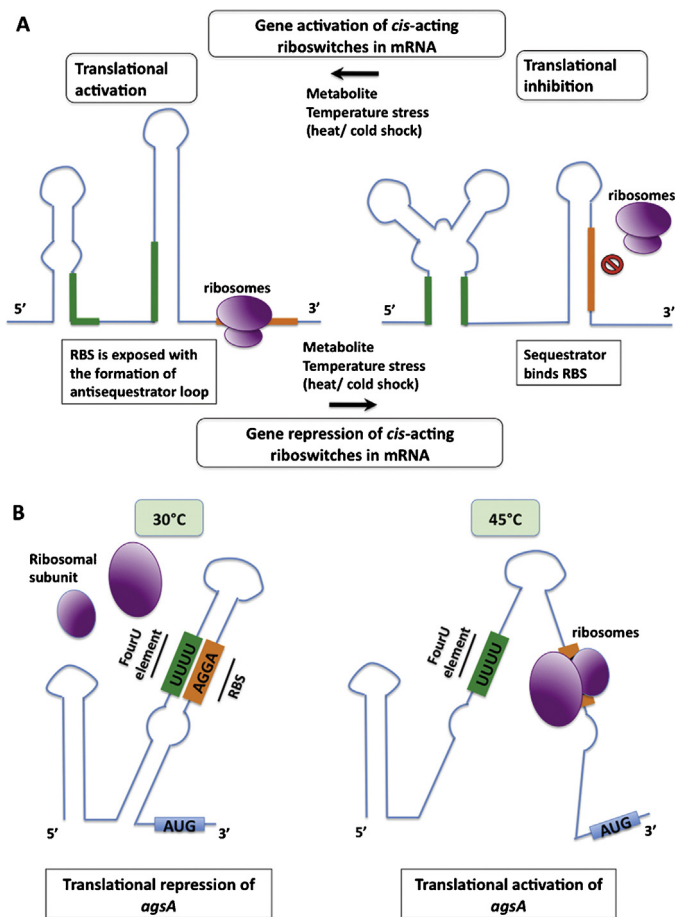
The total RNome of a cell consist of two classes of RNAs: the RNAs that are translated into protein (messenger RNAs, or mRNAs) and various types of RNA that are not translated into protein but are involved in cell regulatory functions (non-protein coding RNAs, or npcRNAs). These regulatory RNAs are also often referred to as small non-messenger RNAs (snmRNAs), small non-coding RNAs (ncRNAs), untranslated RNAs (utrRNAs), small RNAs (sRNAs), or non-protein-coding RNAs (npcRNAs), as they do not contain sizeable open reading frames (ORFs) (Brosius and Tiedge, 2004; Chinni et al., 2010; Davis et al., 2005; Heidrich et al., 2006; Hüttenhofer et al., 2002; Tang et al., 2002; Tjaden et al., 2006; Vogel et al., 2003). Throughout this review, the term sRNAs will be used.

Together with two-component signal transduction and regulatory proteins, sRNAs have been implicated in integrating environmental stress signals and regulating a plethora of stress responses. To date, numerous sRNAs have been identified and predicted in a wide range of bacteria (Abu-Qatouseh et al., 2010; Altuvia, 2007; Pichon and Felden, 2008; Raabe et al., 2011), including those associated with bacterial ribosomal protein operons (Khayrullina et al., 2012). The best characterized class of sRNAs acts via antisense base pairing with target mRNAs or in partnership with protein, forming ribonucleoprotein (RNP). Based on their genomic location, sRNAs can be subdivided into *cis*-encoded sRNAs, which map to the strand of DNA complementary to their mRNA targets, and *trans*-encoded sRNAs, which are located remote from their mRNA target and often exhibit only partial complementarity to them (Richards and Vanderpool, 2011). *Trans*-encoded regulatory RNAs usually require the Hfq chaperone protein as a cofactor to facilitate the interaction between sRNAs and target mRNAs (Sonnleitner et al., 2004; Valentin-Hansen et al., 2004). Hfq is a 102 amino acid protein that was first identified as a host factor required for phage Q $\beta$  RNA replication in *Escherichia coli* (Franze de Fernandez et al., 1968). It preferentially binds to an AU-rich single stranded region upstream of the transcription terminator (Geissmann et al., 2006; Møller et al., 2002; Zhang et al., 1998). Conserved in a wide range of bacteria, Hfq is involved in modulating myriad cellular functions, including stress response. Apart from affecting the activity of transcription factors  $\sigma^E$  and  $\sigma^S$  (Figueroa-Bossi et al., 2006; Muffler et al., 1996), inactivation of the *hfq* gene was found to seriously impair the pathogenicity of uropathogenic *E. coli*, enterohaemorrhagic *E. coli*,

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**Fig. 1.** General mechanism deployed by sRNAs when encountering temperature stress. (A) Depending on the fluctuations in metabolite concentration, pH, or temperature, *cis*-acting riboswitches can adopt alternative structures. In general, conformational changes in structure modulate the availability or sequestration of the ribosome binding site (RBS) and hence activate or inhibit translation. (B) The mRNA of *agsA* is post-transcriptionally regulated by an RNA thermometer in the 5'-UTR. At low temperature (30 °C), ribosome binding is inhibited due to base pairing between the fourU element and the RBS. When the temperature is upshifted to 45 °C, the inhibitory structure is destabilized via a zipper-like mechanism, thus permitting the binding of a ribosomal subunit (purple) as a first step of translational initiation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

*Vibrio cholerae*, *Brucella abortus*, *Legionella pneumophila*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Francisella tularensis*, *Burkholderia cepacia*, *Shigella sonnei*, and *S. flexneri* (Christiansen et al., 2004; Ding et al., 2004; Kadzhaev et al., 2009; Kendall et al., 2011; Kulesus et al., 2008; McNealy et al., 2005; Meibom et al., 2009; Mitobe et al., 2009; Robertson and Roop, 1999; Sharma and Payne, 2006; Sittka et al., 2007; Sonnleitner et al., 2003; Sousa et al., 2010).

Other classes of sRNAs act by binding to proteins and by modulating their activity. In addition, 5'-untranslated regions (UTRs) of mRNAs have been shown to act as riboregulators of bacterial gene expression (e.g., during the stress response) (Fig. 1). In this review, we aim to emphasize the involvement of sRNAs in orchestrating selected stress responses in bacteria (Table 1). Mechanistic details of sRNA action are also reviewed.

### sRNAs in temperature stress

Temperature is one of the most important parameters that bacterial cells need to closely monitor. Often, signal transduction systems featuring complex feedback loops are responsible for

reactions to temperature fluctuations. In contrast to signal transduction system responses that usually have a lag period, RNA-based feedback to temperature fluctuations is more rapid and usually is exerted via changes in structural conformation of regulatory RNA regions termed "RNA thermometers". Frequently, temperature fluctuations affect expression of heat shock, cold shock, and virulence genes. Hence, it is not surprising that these classes of genes employ RNA thermometers to control translation initiation in response to the surrounding temperature.

Typically, RNA thermometers are located within the 5'-UTR of an mRNA and form a secondary structure that occludes the Shine-Dalgarno (SD) sequence from binding to the 30S ribosome subunit (i.e., the switch off state) at low temperature. As the temperature gradually rises, the secondary structure is destabilized via a zipper-like mechanism and formation of translational initiation complexes is permitted (i.e., the switch on state) (Fig. 1A) (Kortmann and Narberhaus, 2012). The most common RNA thermometer is the Repression Of the heat Shock gene Expression (ROSE) element, which is always associated with genes encoding small heat shock proteins. ROSE elements have been found in rhizobia (Nocker et al., 2001a,b) and  $\alpha$ - and  $\gamma$ -proteobacteria (Waldminghaus et al., 2005). A conserved short stretch of nucleotides (U(U/C)GCU) in close proximity to the SD sequence serves as a signature motif for ROSE elements. Ranging from 60 to >100 nucleotides in length, ROSE elements are predicted to possess two, three, or four stem-loops. The 5' hairpins often remain stable under heat shock conditions, whereas the 3' hairpin that contain the SD sequence is only formed at low temperature (Waldminghaus et al., 2009). Increased temperature induces melting of the 3'-RNA stem-loop structure, which in turn results in the facilitation of ribosome binding to the SD sequence and therefore activation of mRNA translation.

Another class of RNA thermometers is the fourU element. The fourU thermometer was first identified in the 5'-UTR of aggregation suppressing A (*agsA*) mRNA of *Salmonella* (Waldminghaus et al., 2007). The expression of AgsA protein is regulated in a temperature-dependent manner. Similar to ROSE elements, the fourU thermometers form hairpin structures that include base pair interaction between four uridines and AGGA nucleotides of the SD sequence in one of the helices at low temperature (Fig. 1B). During heat shock conditions, the fourU-SD helix is destabilized, thus permitting translation. The unique features of these sophisticated RNA thermometers are yet to be defined. FourU elements are also found in 5'-UTRs of several heat shock and virulence genes in *Salmonella* (Waldminghaus et al., 2007).

RNA thermometers are also crucial in the cold shock response. The cold shock response results in the production of several proteins that counteract membrane rigidification and in the formation of stable RNA structures that interfere with translation initiation and elongation. For instance, cold shock protein A (CspA) is an RNA chaperone that binds single stranded RNAs to prevent formation of secondary structures at cold temperatures (Jiang et al., 1997). CspA has been reported to function as an anti-terminator of transcription, and it facilitates transcriptional read-through of several genes induced during cold shock (Bae et al., 2000). The expression of the *cspA* gene is upregulated at low temperature due to the specific RNA thermometer located in the 5'-UTR of *cspA* mRNA. Unlike the heat shock RNA thermometer, the *cspA* 5'-UTR forms mutually exclusive alternative structures, thus increasing mRNA stability and translation at 10 °C compared to 37 °C (Fig. 1A) (Giuliodori et al., 2010).

In addition, a *trans*-encoded sRNA, DsrA, which is expressed abundantly in *E. coli* at low temperature, acts as an RNA thermometer and positive regulator for the stress induced sigma factor RpoS regulon (Repoila and Gottesman, 2003; Sledjeski et al., 1996). In *E. coli*, DsrA is present as full-length transcript (F form) and truncated transcript (T form). The ratio between the two forms of DsrA

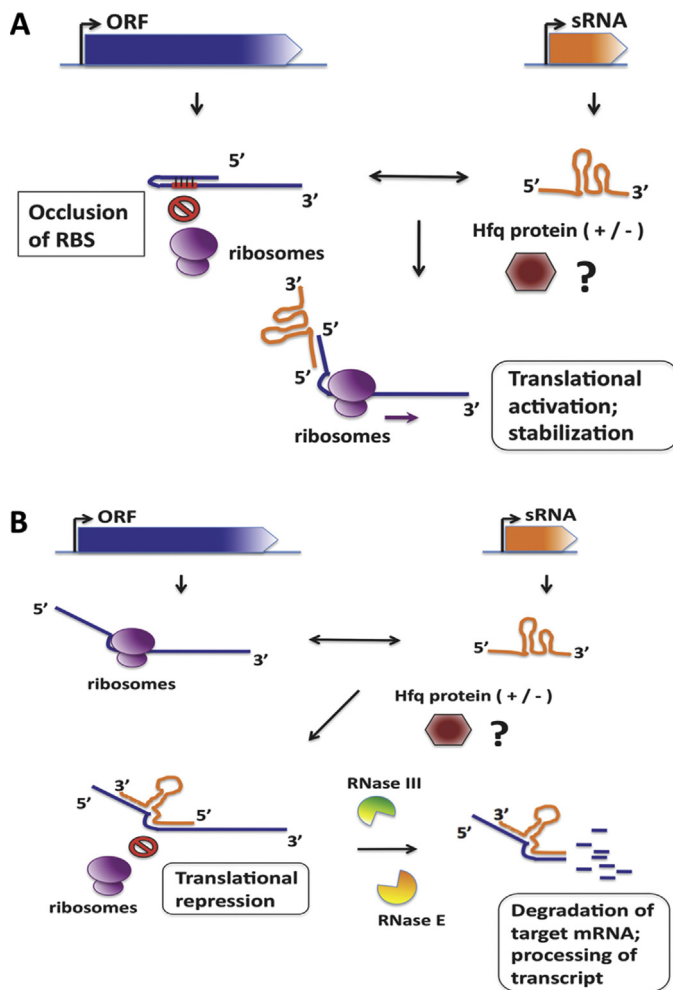
**Table 1**  
Selected sRNAs induced upon selected stress conditions in model microorganisms.

sRNA	Bacteria	Type of sRNA/regulatory mechanism	Phenotype/response	References
<b>Temperature stress</b>				
Upstream of <i>ibpAB</i>	<i>E. coli</i>	ROSE element of RNA thermometer	Translationally active at 42 °C. Stabilizes denatured protein for refolding.	Gaubig et al. (2011)
Upstream of <i>agsA</i>	<i>S. typhimurium</i>	FourU element of RNA thermometer	Translational initiation at 45 °C but not at 30 °C. Virulence.	Waldminghaus et al. (2007)
Stem I and stem III of <i>rpoH</i>	<i>E. coli</i>	RNA thermometer	Temperature upshift to 42 °C increases translational efficiency and stability of RpoH ( $\sigma^H$ ).	Morita et al. (1999a,b)
Upstream of <i>prfA</i>	<i>L. monocytogenes</i>	RNA thermometer	Translationally active at 37 °C but not 30 °C. Virulence.	Johansson et al. (2002)
Upstream of <i>cspA</i>	<i>E. coli</i>	RNA thermometer	Structural rearrangement yields a translationally active structure at 10 °C but not 37 °C. Cold shock protection.	Giuliodori et al. (2010)
<b>Metabolite/nutrient stress</b>				
SgrS	<i>E. coli</i> ; <i>S. typhimurium</i>	Trans-encoded sRNA	Activated by SgrR. Degrades <i>ptsG</i> mRNA to relieve glucose phosphate stress; degrades <i>manXYZ</i> mRNA under stress; suppresses the synthesis of SopD.	Papenfort et al. (2012), Rice and Vanderpool (2011) and Vanderpool and Gottesman (2004)
CsrB, C	<i>E. coli</i>	Protein binding sRNA	Induced upon amino acid starvation to repress CsrA.	Jonas and Melefors (2009)
RsaE	<i>S. aureus</i>	Trans-encoded sRNA	Down-regulates key enzymes of central metabolism under carbon starvation.	Bohn et al. (2010) and Geissmann et al. (2009)
SdsR	<i>S. typhimurium</i>	Trans-encoded sRNA. Hfq-dependent	Activated by $\sigma^S$ . Down-regulates <i>ompD</i> mRNA under nutrient deprived condition.	Fröhlich et al. (2012)
GcvB	<i>E. coli</i> ; <i>S. typhimurium</i>	Trans-encoded sRNA. Hfq-dependent	Activated by GcvA when cellular glycine is high; self-repressed by GcvA and GcvR when cellular glycine is low. Represses genes involved in the transport of small peptides and polar and branched amino acids.	Pulvermacher et al. (2009a,b, 2008), Sharma et al. (2011, 2007), Stauffer and Stauffer (2012) and Urbanowski et al. (2000)
<b>Envelope/outer membrane proteins (OMPs) stress</b>				
MicA	<i>E. coli</i> ; <i>S. typhimurium</i>	Trans-encoded sRNA. Hfq-dependent	Binding to Translation Initiation Region (TIR) of <i>ompA</i> , thus occluding RBS of <i>ompA</i> .	Rasmussen et al. (2005) and Udekwi et al. (2005)
RybB	<i>E. coli</i> ; <i>S. typhimurium</i>	Trans-encoded sRNA. Hfq-dependent	Down-regulates multiple major <i>omp</i> mRNAs in <i>S. typhimurium</i> ; Down-regulates <i>ompC</i> and <i>ompW</i> mRNA in <i>E. coli</i> .	Papenfort et al. (2010), Johansen et al. (2006), Papenfort et al. (2006)
CyaR	<i>E. coli</i> ; <i>Salmonella</i>	Trans-encoded sRNA. Hfq-dependent	Activated by cAMP. Down-regulates <i>ompX</i> mRNA.	De Lay and Gottesman (2009), Johansen et al. (2008) and Papenfort et al. (2008)
VrrA	<i>V. cholerae</i>	Trans-encoded sRNA. Requirement of Hfq varies.	Binds to the 5' region of <i>ompA</i> mRNA and represses translation of <i>ompA</i> , thus promotes release of OMV; Down-regulates TCP and OmpT.	Song et al. (2010, 2008)
<b>Oxidative stress</b>				

Table 1 (Continued)

sRNA	Bacteria	Type of sRNA/regulatory mechanism	Phenotype/response	References
OxyS	<i>E. coli</i>	Trans-encoded sRNA. Hfq-dependent for translational repression of <i>rpoS</i>	Represses the translation of <i>fhlA</i> via formation of kissing complex; Binds to <i>rpoS</i> mRNA and represses the translation of <i>rpoS</i> .	Altuvia et al. (1998, 1997), Argaman and Altuvia (2000) and Zhang et al. (1998)
Aspks	<i>M. tuberculosis</i>	Cis-encoded sRNA	Represses the <i>pkc8</i> , <i>pkc12</i> , and <i>pkc15</i> mRNA for survival in macrophages?	Arnvig and Young (2009) and Schnappinger et al. (2003)
IsrC, N	<i>S. typhimurium</i>	Trans-encoded?	Similar time-course expression to OxyS upon infection in macrophages.	Padalon-Brauch et al. (2008)
<b>Iron deficiency stress</b>				
RyhB	<i>E. coli</i> ; <i>S. dysenteriae</i> ; <i>K. pneumoniae</i> ; <i>V. cholerae</i>	Trans-encoded? Hfq-dependent?	Repression of virulence related genes. Activates iron scavenging related genes. Biofilm formation? Chemotaxis?	Davis et al. (2005), Huang et al. (2012), Massé and Gottesman (2002), Massé et al. (2005), Murphy and Payne (2007) and Prévost et al. (2007)
PrrF1, F2	<i>P. aeruginosa</i>	Trans-encoded? Hfq-dependent?	Represses translation of mRNA for iron-using protein and anthranilate, a precursor for Pseudomonas quinolone signal (PQS). Similar function as PrrF1, F2?	Oglesby et al. (2008) and Wilderman et al. (2004)
PrrH	<i>P. aeruginosa</i>	Overlap PrrF1, F2		Oglesby-Sherrouse and Vasil (2010)
NrrF	<i>N. meningitidis</i>	Trans-encoded sRNA. Hfq-dependent	Represses translation of <i>sdhCDAB</i> .	Mellin et al. (2007) and Metruccio et al. (2009)
FsrA	<i>B. subtilis</i>	Trans-encoded sRNA. Hfq-independent	Represses translation of <i>sdhABC</i> and other iron-using proteins.	Gaballa et al. (2008)
<b>pH stress</b>				
GadY	<i>E. coli</i>	Cis-encoded sRNA. Hfq-independent	Binding to 3'-end of <i>gadX</i> confers protection from RNase degradation, thus increases expression of downstream acid resistance genes.	Opdyke et al. (2004)
GcvB	<i>E. coli</i>	Trans-encoded sRNA	Increases translation of <i>rpoS</i> to survive low pH?	Jin et al. (2009)
PRE- <i>alx</i>	<i>E. coli</i>	pH-responsive riboswitch	Translational active structure of <i>alx</i> is formed under high pH.	Nechooshtan et al. (2009)
<b>Anaerobic stress</b>				
FnrS	<i>E. coli</i>	Trans-encoded sRNA. Hfq-dependent	Activated by FNR. Represses translation of many genes encoding enzymes with "aerobic" functions or enzymes linked to oxidative stress using a different region of the sRNA.	Boysen et al. (2010) and Durand and Storz (2010)
AniS	<i>N. meningitidis</i>	Trans-encoded sRNA. Hfq-dependent and Hfq is involved in turnover of AniS	Activated by FNR. Represses NMB0214 (encoding PrlC oligopeptidase) and NMB1468 (encoding a lipoprotein)?	Fantappiè et al. (2011)
PhrS	<i>P. aeruginosa</i>	Trans-encoded sRNA. Hfq-dependent	Activated by ANR. PhrS base pairs with 5'-leader of <i>pqsR</i> mRNA, resulting in structural rearrangements and translational activation of <i>pqsR</i> mRNA, thus increases in PQS and PYO. Quorum-sensing? Virulence?	Sonnleitner et al. (2011)





**Fig. 2.** General mechanism of *trans*-encoded sRNAs when encountering stress. (A) *Trans*-encoded sRNAs can bind to target mRNAs and relieve the occlusion of the RBS, thus allowing translation initiation. Hfq is usually involved as the binding facilitator between sRNA and target mRNA. (B) By base pairing *trans*-encoded sRNAs can also occlude the RBS and repress translation. Usually the non-translated mRNA is degraded via an RNase E-dependent pathway. The interaction between sRNA and target mRNA can also induce or unmask sites for RNase III. This usually leads to degradation of both mRNA and sRNAs. Involvement of Hfq varies from case to case.

(F/T) sRNA varies in a temperature-dependent manner, with relatively more T formed at higher temperatures. At low temperature, the F form of DsrA outnumbers the T form. Thus, the 5' region of the F form interacts via RNA:RNA base pairing with the leader sequence of the *rpoS* mRNA, which leads to structural changes in its 5'-UTR and activation of mRNA translation (Fig. 2A) (Repoila and Gottesman, 2001).

RNA thermometers are also exploited as regulators of virulence gene expression. This function is perhaps best documented by the virulence gene activator PrfA of *L. monocytogenes* (Johansson et al., 2002). *L. monocytogenes* survives primarily as a saprophyte in soils but is capable of transitioning into a pathogen upon ingestion by a susceptible human host (Freitag et al., 2009). Hence, 37 °C constitutes temperature stress and is indicative of invasion into the mammalian host. At this temperature, the 127 nucleotides long secondary structure in the 5'-UTR of *prfA* is destabilized and *prfA* is effectively translated (Johansson et al., 2002). This results in expression of several virulence genes that encode phagosome-escape factors, adhesins, and several immune modulating factors that repress the host innate immune response (Freitag et al., 2009).

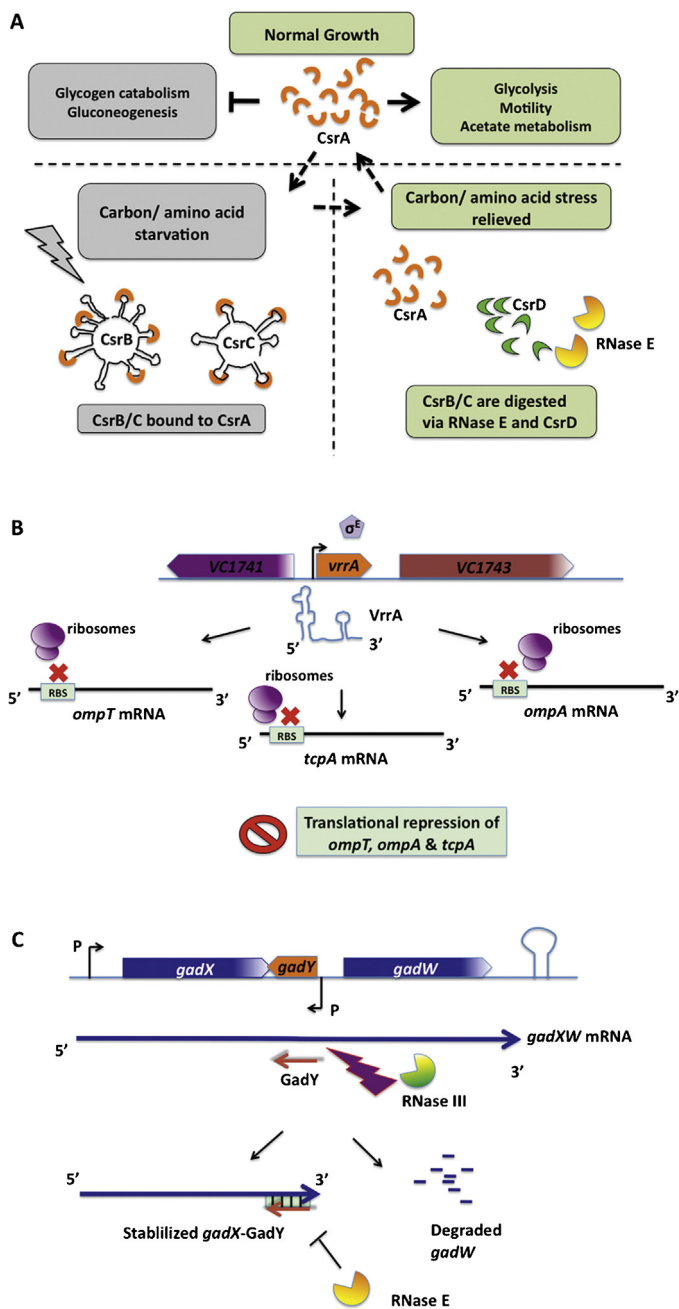
## sRNAs in metabolite/nutrient stress

Microbes are most frequently found in a state of nutrient deprivation that leads to slow growth or stasis. During glucose-phosphate stress, accumulation of glucose-6-phosphate (G6P) or the non-metabolizable glucose analog  $\alpha$ -methyl-glucoside 6-phosphate ( $\alpha$ MG6P) occurs, which would lead to growth arrest (Englesberg et al., 1962) and cell death (Irani and Maitra, 1977). In *E. coli*, both glucose and  $\alpha$ -methyl-glucoside ( $\alpha$ MG) are transported into the cell and concomitantly phosphorylated by the phosphoenolpyruvate phosphotransferase system (PTS). The EIICB<sup>Glc</sup> (PtsG) transporter encoded by the *ptsG* gene is one of the main glucose transporters of the phosphoenolpyruvate PTS. Under glucose-phosphate stress, an Hfq-associated sRNA, SgrS (previously known as RyaA), negatively regulates *ptsG* mRNA at the post-transcriptional level, preventing new synthesis of the PtsG protein and further accumulation of G6P or  $\alpha$ MG6P (Kawamoto et al., 2006; Vanderpool and Gottesman, 2004). This is achieved by specific base pairing between SgrS and *ptsG* mRNA, which results in translational inhibition and subsequent degradation of the *ptsG* transcript in an RNase E-dependent manner (Fig. 2B) (Maki et al., 2010; Morita et al., 2005). SgrS is also reported to encode a small peptide, SgrT, which inhibits PtsG activity by unknown mechanisms (Wadler and Vanderpool, 2007). A recent study highlighted another PTS, ManXYZ, also regulated by SgrS at the post-transcriptional level in a manner akin to that of PtsG under glucose-phosphate stress (Rice and Vanderpool, 2011). Additionally, SgrS has been shown to suppress the synthesis of SopD, a *Salmonella*-specific virulence protein, at the post-transcriptional level (Papenfert et al., 2012). This finding suggests that an interesting link between glucose-phosphate stress and virulence factors may exist.

The central player of the carbon storage regulatory (Csr) system, CsrA, has been shown to directly down-regulate *cstA* gene expression during carbon starvation (Fig. 3A) (Dubey et al., 2003). Moreover, CsrA protein can also regulate biofilm formation and motility in bacteria. In *E. coli*, CsrA activity is indirectly mediated by a two-component system (BarA/UvrY) during carbon starvation. BarA is the sensor protein and UvrY is the cognate response regulator. The UvrY protein activates the expression of two sRNAs, CsrB and CsrC, which can specifically bind multiple copies of the CsrA protein. This binding concomitantly results in repression of glycolysis and activation of gluconeogenesis (Fig. 3A) (Pernestig et al., 2003). Both sRNAs harbor imperfect repeat sequences (9 in CsrC and 18 in CsrB) that form stem-loop structures that mimic the CsrA binding sites on its mRNA targets. The sRNA expression is rapidly decreased upon addition of casamino acids, tryptone, or a mixture of pure amino acids into minimal medium and is induced upon amino acid starvation (Jonas and Meleforts, 2009). The stability of CsrB and CsrC is regulated by another protein, CsrD, via an RNase E-mediated pathway (Fig. 3A) (Suzuki et al., 2006).

In *Caulobacter crescentus*, CrfA sRNA is induced upon carbon starvation and entry into the stationary phase. CrfA contains a region that is predicted to hybridize to a stem-loop structure upstream of the putative TonB-dependent receptor (CC3461) ribosome binding site (RBS), resulting in mRNA stabilization against ribonucleases degradation (Fig. 2A). Hence, import of a greater variety of potential carbon sources is achievable through CC3461 mRNA accumulation, resulting in an increase of TonB-dependent receptor production (Landt et al., 2010).

RsaE is another sRNA induced at the late exponential growth stage when cells are deprived of nutrients. RsaE is conserved in *Staphylococcus*, *Micrococcus*, and *Bacillus* and forms two stem-loop structures separated by a 17 nucleotides single stranded region. Based on RNA:RNA interaction, RsaE can prevent the formation of the ribosomal initiation complex on two mRNA targets within the same operon: *opp3B*, the first mRNA of the *opp3* operon (Geissmann



**Fig. 3.** Strategies employed by bacterial sRNAs in response to selected stress conditions. (A) Under normal growth conditions, the CsrA protein favors activation of genes involved in glycolysis while impeding the activation of genes involved in gluconeogenesis. CsrB and CsrC RNAs will bind to the CsrA protein under carbon starvation and suppress the activity of CsrA. When carbon starvation is relieved, CsrB and CsrC are degraded by RNase E-mediated mechanism via CsrD protein. (B) VrrA is a *trans*-encoded sRNA mapping between two genes, *vc1741* and *vc1743*. Induced upon envelope stress and under the direct regulation of transcription factor  $\sigma^E$ , VrrA binds to the RBS of *ompA*, *ompT*, and *tcpA* and impedes binding of the 30S ribosomal subunit. Thus, suppression of OmpA, OmpT, and TcpA synthesis is achieved. (C) When the *cis*-encoded GadY sRNA is present upon exposure to low pH, the *gadX*-*gadW* bicistronic mRNA is processed to give rise to two RNAs. GadY is complementary to the 3'-end of the *GadX* mRNA. Part of this processing is due to GadY-dependent RNase III cleavage. This results in stabilization of *gadX* and degradation of *gadW* under acidic conditions.

et al., 2009), and *opp3A*, the last mRNA of the *opp3* operon (Fig. 2B) (Bohn et al., 2010). In concert with down-regulation of key enzymes in the TCA cycle and purine biosynthesis, RsaE assists *S. aureus* in adapting to nutrient-deprived conditions (Bohn et al., 2010).

Metabolism of amino acids also must be accurately regulated due to their imperative roles in serving as substrates for protein synthesis as well as precursors for other cellular constituents such as nucleotides and enzyme cofactors. For example, the glycine cleavage (*Gcv*) operon, *gcvTHP*, is essential in catalyzing the oxidative cleavage of glycine to ammonia, carbon dioxide, and 5,10-methylenetetrahydrofolate and provides a secondary pathway for one-carbon biosynthesis (Kikuchi, 1973; Meedel and Pizer, 1974). Expression of *gcvTHP* and another Hfq-associated sRNA, GcvB, is activated by the GcvA protein when the cellular glycine level is high; when cellular glycine is scarce, GcvB is repressed by GcvA and another protein, GcvR (Urbanowski et al., 2000). In *E. coli* and *Salmonella*, GcvB represses genes involved in the transport of small peptides, polar and branched amino acids, as well as other toxins and antibiotics. Hence repression by GcvB can prevent transport of toxic compounds into the cell (Pulvermacher et al., 2008, 2009a,b; Sharma et al., 2007, 2011; Urbanowski et al., 2000). Interestingly, GcvB sRNA contains two antisense elements that can regulate mRNA targets. It was suggested that more than 1% of all *Salmonella* genes were regulated via conserved G/U-rich element of GcvB. However, *cycA* mRNA of *S. enterica* and *E. coli* could be regulated by both antisense elements and by additional small regions within GcvB, indicating a high degree of flexibility in base pairing with target mRNAs (Sharma et al., 2011; Stauffer and Stauffer, 2012). This interesting feature suggests that more target mRNAs could be discovered in near future.

### sRNAs involved in envelope/outer membrane protein (OMP) stress

In Gram-negative bacteria, the outer membrane (OM) forms the bacterial cell envelope together with the peptidoglycan layer and the inner membrane. The OM prevents the entry of many toxic molecules into the cell and ensures bacterial survival in hostile environments. Porins form channels through the OM for the uptake of nutrients and the excretion of waste compounds. For instance, *E. coli* OmpF and OmpC are abundant proteins that form the trimeric  $\beta$ -barrels in the OM. In addition, outer membrane proteins (OMPs) also function as enzymes, adhesins, and bacterial surface proteins, which often confer bacterial virulence. Hence, it is not surprising that the expression of OMPs is under complex regulation (Nikaido, 2003).

In *E. coli* and *Salmonella*, the folding status of OMPs is monitored by the  $\sigma^E$  signaling system, which is required both under stress and normal growth conditions. In response to accumulation of unfolded and/or misfolded porins as a result of envelope stress, the  $\sigma^E$  system up-regulates the expression of genes encoding factors that will counteract the damage (Alba and Gross, 2004; Ruiz and Silhavy, 2005). In the absence of cell envelope stress,  $\sigma^E$  is sequestered by the inner membrane-bound protein, RseA. Envelope stress promotes RseA degradation, which occurs by a proteolytic cascade initiated by DegS protease, leading to the release of  $\sigma^E$  and activation of  $\sigma^E$ -dependent genes (Ades, 2004; Alba and Gross, 2004; Ruiz and Silhavy, 2005).

OmpA protein is highly conserved among enterobacteria. Throughout bacterial growth the *ompA* mRNA is abundant and has a relatively long half-life. However, RNA levels drastically drop when the bacteria enter the stationary phase (Nilsson et al., 1984). It has been demonstrated that this effect is mediated by the Hfq-dependent *trans*-encoded regulatory sRNA, MicA (formerly denoted as SraD) (Rasmussen et al., 2005; Udekwi et al., 2005). A 17 nucleotides stretch in MicA forms a nearly perfect duplex with the 5'-UTR of *ompA* mRNA, thereby masking its SD sequence. The resulting RNA duplex is subjected to RNase E-mediated decay (Fig. 2B). The  $\sigma^E$ -dependent decrease of *ompA* mRNA levels is

abolished in bacterial mutants with *micA* deletion (Rasmussen et al., 2005; Udekwu and Wagner, 2007; Udekwu et al., 2005). Similarly, Papenfort et al. (2010) showed that multiple *omp* mRNAs were down-regulated in a  $\sigma^E$ -dependent pathway by another sRNA, RybB (Fig. 2B). Since both MicA and RybB are conserved among enterobacteria (Johansen et al., 2006), it infers their importance in regulating the homeostatic loop that prevents the flux of OMPs to the envelope under envelope stress (Papenfort et al., 2006).

The importance of  $\sigma^E$ -dependent sRNAs in negative regulation of OMPs upon envelope stress was further documented by the identification of CyaR RNA (formerly known as RyeE) in *E. coli* and *S. typhimurium*. CyaR RNA facilitates decay of the porin OmpX encoded mRNA (De Lay and Gottesman, 2009; Johansen et al., 2008; Papenfort et al., 2008). Transcription of the *cyaR* gene, which results in translational initiation of *ompX*, is activated by the cyclic AMP (cAMP) receptor protein CRP when the cAMP level increases (low glucose). It is unclear, however, how the increased levels of  $\sigma^E$  trigger up-regulation of *cyaR* in *E. coli*, but at the same time down-regulate this sRNA in *Salmonella* (De Lay and Gottesman, 2009; Johansen et al., 2008; Papenfort et al., 2008). OmpX has been implicated in promoting bacterial adhesion (Mecses et al., 1995). Since *ompX* is also a target of down-regulation by MicA, down-regulation of *ompX* via CyaR appears to increase metabolic economy, as adhesion may not be a priority when the cell is starved of glucose (Johansen et al., 2008).

Another  $\sigma^E$ -dependent sRNA, VrrA (Fig. 3B) (Song et al., 2008), was shown to base pair with the 5' region of *ompA* mRNA and to repress its translation independently of Hfq in *V. cholerae*, albeit interaction between Hfq and *ompA* mRNA has been reported in *E. coli* (Fig. 3B) (Vytvytska et al., 2000). When OmpA levels decrease in response to repression by VrrA, envelope stress is further relieved by increased production of outer membrane vesicles (OMVs). Similarly, VrrA has also been shown to repress the virulence of *V. cholerae* via suppression of *tcpA*, which encodes toxin co-regulated pilus (Song et al., 2008). In addition, VrrA has been demonstrated to repress *ompT* translation by base pairing with the 5' region of the mRNA in an Hfq-dependent manner in *V. cholerae*, hinting at a complex stress regulation for OMPs (Fig. 3B) (Song et al., 2010).

### sRNAs in oxidative stress

The two best-studied primary signal transduction proteins that regulate adaptive responses to oxidative stress in many bacterial species are OxyR and SoxR. OxyR is a member of the LysR family of transcriptional regulators and activates expression of defensive genes such as those encoding catalases, alkyl hydroperoxide reductases, and superoxide dismutases. OxyR is activated by H<sub>2</sub>O<sub>2</sub> via a reversible thiol-disulfide redox switch (Pomposiello and Demple, 2001). SoxR belongs to the MerR family of transcriptional regulators and is activated by superoxide anion (O<sub>2</sub><sup>•−</sup>)-dependent reversible one-electron oxidation of its iron-sulfur center. Nitric oxide (NO) can also activate SoxR via nitrosylation of iron-sulfur centers with a displacement of sulfide to form dinitrosyl-iron-dithiol(cysteine) complexes (Pomposiello and Demple, 2001).

Exposure of *E. coli* to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) induces transcription of another sRNA, OxyS. As a pleiotropic regulator, OxyS increases and decreases expression of multiple genes and, in part, protects cells from spontaneous and chemically induced mutagenesis. A number of mRNA targets that are regulated by OxyS sRNA have been identified (Altuvia et al., 1997). Among those targets, OxyS has been reported to repress translational initiation of *fhlA* (encoding a transcriptional activator) (Altuvia et al., 1998; Argaman and Altuvia, 2000) and *rpoS* (encoding the  $\sigma^S$  subunit of RNA polymerase) (Zhang et al., 1998) in complex with the Hfq protein (Fig. 2B). However,  $\sigma^S$  also has been demonstrated to be crucial for

bacteria in counteracting the oxidative stress response (Barth et al., 2009). Translational repression of *rpoS* will lead to down-regulation of respiration and superoxide production. This is not surprising, as limitation of the intracellular production of oxygen free radicals is achievable via the repression of some *rpoS*-dependent genes, such as *poxB* (encoding pyruvate oxidase) (Eisenstark et al., 1996), which might leak more oxygen free radicals and thus damage the cell under oxidative stress (González-Flecha and Demple, 1999).

Because pathogenic bacteria invading host macrophages always encounter oxidative stress, it is not surprising that these bacteria exploit sRNAs to survive during the early stages of infection. For instance, B11, B55, F6, and ASps sRNAs are induced in *Mycobacterium tuberculosis* upon H<sub>2</sub>O<sub>2</sub> exposure (Arnvig and Young, 2009), and expression of ASps regulatory RNA coincides with down-regulation of *pks8*, *pks12*, and *pks15* mRNAs (Schnappinger et al., 2003). Determining whether this is a result of direct targeting will require further investigation. IsrC and IsrN sRNAs encoded within genetic islands of *Salmonella typhimurium* also display temporal expression pattern similar to that of OxyS upon infection of macrophage-like cells by *S. typhimurium*. This suggests that IsrC and IsrN could play a crucial role during oxidative stress (Padalon-Brauch et al., 2008).

### sRNAs in iron deficiency stress

Iron is a critical component of cellular activity and is essential for cell growth and differentiation, electron transfer, oxygen transport, metabolism, activation, and detoxification (Kadner, 2005). High intracellular iron levels, however, may be detrimental to bacteria due to the formation of undesired reactive oxygen species (ROS).

In most bacteria, the ferric uptake regulator (Fur) acts as a central sensor and regulator of iron homeostasis. Under iron-rich conditions, it typically represses iron uptake genes such as siderophores while genes encoding iron-containing and iron-storage proteins are expressed; however, Fur becomes inactive at low intracellular iron, while iron uptake genes are expressed (Hantke, 2001). Fur also is involved in dealing with multiple cellular functions, such as oxidative stress, energy metabolism, acid tolerance, and virulence gene production (Bijlsma et al., 2002; Hassett et al., 1996; Ochsner and Vasil, 1996; Van Vliet et al., 2003).

RyhB was first reported in *E. coli* as an sRNA expressed under iron starvation conditions, and it is controlled by the Fur protein. *ryhB* is transcriptionally repressed by Fur and the Fe<sup>2+</sup> (Fur-Fe<sup>2+</sup>) complex. Its expression results in decreased mRNA levels of six genes during iron starvation: *sdhCDAB* (encoding succinate dehydrogenase), *acnA* (encoding aconitase), *fumA* (encoding fumarase), *bfr* and *ftnA* (both encoding ferritins), and *sodB* (encoding superoxide dismutase) (Massé and Gottesman, 2002). In another microarray study, Massé et al. (2005) showed that RyhB down-regulated at least 18 bacterial operons encoding a total of 56 proteins that are mostly involved in iron metabolism. However, repression of *ftnA* was reported to be RyhB independent (Massé et al., 2005) and was indeed suppressed by the histone-like nucleoid-associated protein (H-NS) (Nandal et al., 2010). Notably, RyhB sRNA also exerts feedback by repressing *fur* mRNA translation, thus regulating the Fur protein level (Večerek et al., 2007).

Because iron homeostasis is involved in global regulatory networks, it is not surprising that RyhB can regulate multiple targets. Indeed, RyhB inhibits translation initiation and stimulates *cysE* mRNA degradation to increase the serine flux into enterobactin biosynthesis instead of cysteine biosynthesis during iron starvation (Salvail et al., 2010). RyhB also base pairs with the 5' UTR of *shiA* mRNA to displace an intrinsic inhibitory structure that sequesters the ribosome binding site and thus permits formation of the translation initiation complex; thereby increasing translation



and stability of the mRNA encoding shikimate permease protein (Fig. 2A). This protein is essential in the production of a high affinity siderophore (an enterochelin that acquires iron for bacteria) (Prévost et al., 2007). RyhB also is involved in regulation of the operons that encode proteins participating in Fe-S cluster formation. This is achieved via RyhB-targeted degradation of the *iscSUA* polycistronic transcript from the *iscRSUA* operon. The *iscRSUA* operon encodes proteins that are involved in Fe-S cluster formation under normal growth conditions. The sRNA exhibits partial complementarity to the intergenic (UTR) region between *iscR* and *iscS* mRNAs of the *iscRSUA* transcript. The presence of a stable secondary structure in the 3'-UTR of *iscR* mRNA protects the RNA from further degradation during RNase E-dependent cleavage of the *iscSUA* polycistronic RNA. The resulting accumulation of the transcriptional regulator IscR activates the *suf* operon that encodes proteins involved in Fe-S cluster synthesis during iron starvation (Desnoyers et al., 2009).

RyhB homologs have been identified in *V. cholerae*, *S. flexneri*, and *S. dysenteriae*. Broadening roles for RyhB have been proposed in processes such as biofilm formation and chemotaxis (in *V. cholerae*) (Davis et al., 2005; Mey et al., 2005), acid resistance reduction (in *S. flexneri*) (Oglesby et al., 2005), and suppression of virulence genes (in *S. dysenteriae*) (Murphy and Payne, 2007). RyhB-like mechanisms also have been identified in other bacterial species. For instance, two sRNAs, PrrF1 and PrrF2, in *P. aeruginosa* are induced upon iron depletion. Induced PrrF1 and PrrF2 led to a decrease in mRNAs related to iron-utilizing proteins (Wilderman et al., 2004) and to repression of anthranilate, a precursor for the *Pseudomonas* quinolone signal (PQS) (Oglesby et al., 2008). Oglesby-Sherrouse and Vasil (2010) recently reported a longer sRNA, PrrH (325 nucleotides), that is expressed during the heme-modulated response and harbors sequences of both PrrF1 and PrrF2 RNA within its structure. Identification of RyhB homologs such as NrrF (Mellin et al., 2007; Metruccio et al., 2009) and FsrA (Gaballa et al., 2008) in *Neisseria meningitidis* and *Bacillus subtilis*, respectively, have shed further light on iron homeostasis networks in bacterial physiology.

### sRNAs in pH stress

The ability of enteric bacteria to thrive in the extremely acidic environment of the stomach is crucial for colonization of the intestine. In several Gram-positive and Gram-negative bacteria, the glutamate-based acid resistance system (gad system) is a key factor in the protection of the cell from the deleterious effects of high proton concentrations (Castanie-Cornet et al., 1999; Foster, 2001). Two glutamate decarboxylase enzymes, GadA and GadB, catalyze the production of  $\gamma$ -aminobutyric acid using glutamate as the substrate, whereas GadC is a putative amino acid antiporter that exchanges  $\gamma$ -aminobutyric acid for external glutamate. GadE, GadX, GadW, CRP, nucleoid protein H-NS, and  $\sigma^S$  regulate the expression of gad-related enzymes in a complex manner (Ma et al., 2003a, 2003b; Shin et al., 2001; Tramonti et al., 2002).

In *E. coli*, Hfq-dependent GadY sRNA (105 nucleotides) maps to the intergenic region between *gadX* and *gadW* and is transcribed in *cis*-antisense orientation overlapping the 3'-UTR of the *gadX* mRNA (Fig. 3C). In response to acid stress, GadY initially was reported to bind to the 3'-UTR of *gadX*, thus protecting the mRNA from RNase E degradation by formation of an RNA duplex (Opdyke et al., 2004). It was then reported that targeting of the intergenic region in the *gadX-gadW* operon by GadY sRNA could induce RNase III-mediated cleavage of the bicistronic *gadXW* transcript (Fig. 3C). Operon processing in combination with stabilization of the *gadX* mRNA by GadY sRNA leads to accumulation of the transcriptional regulator protein GadX and increased expression of downstream acid resistance genes for survival at low pH (Opdyke et al., 2011). GcvB sRNA also has been reported to enhance the ability of *E. coli*

to survive at low pH by up-regulating the levels of the  $\sigma^S$  via an as yet unknown mechanism (Jin et al., 2009).

Prokaryotes utilize numerous methods to deal with the high cytoplasmic pH, including increased acid production via amino acid deaminases and sugar fermentation, changes in the properties of the cell surface, and increased activity of transporters (Padan et al., 2005). Appealingly, the 5'-UTR of *alx* mRNA has been reported to serve as a pH responsive RNA element (PRE riboswitch) that activates production of the Alx (putative transporter) protein under alkaline conditions, but not at neutral pH. During normal growth, the PRE region forms a translationally inactive structure (structure N) by occluding the SD sequence and the translational start codon in a hairpin structure. Under extremely alkaline conditions, short pausing of RNA polymerase occurs within the PRE region. The pausing signals are located upstream from the SD motif and lead to the formation of an alternative structure (active structure H) in the newly synthesized 5'-UTR of *alx* mRNA. This RNA structure does not encompass the untranscribed sequence of the ribosomal binding site and the translational start codon. Upon further elongation of transcription, the synthesized SD region is structurally open for binding of the 30S ribosomal subunit, and initiation of translation ensues. Because conservation of this riboregulator has been noted among enteric bacteria (Nechooshtan et al., 2009), discoveries of other pH responsive riboregulators in other bacteria are anticipated.

### sRNAs in oxygen stress/anaerobic conditions

The bacterial adaptive response to anaerobic or anoxic surroundings is mediated by two global regulators: the fumarate and nitrate reduction (FNR) protein and the aerobic respiratory control (Arc) two-component system, which consists of the ArcA response regulator and the ArcB sensor kinase. In response to anaerobiosis, FNR directly senses for oxygen ( $O_2$ ) scarcity via the acquirement of a  $[4Fe-4S]^{2+}$  cluster that permits FNR dimerization. The ArcA/B pair indirectly senses  $O_2$  deprivation via reduction of oxidized quinone, which leads to autophosphorylation of ArcB and activation of ArcA (Crack et al., 2012; Green et al., 2009). FNR activation leads to repression of genes with aerobic functions and activation of genes encoding anaerobic pathway enzymes. In contrast, the activation of ArcA results in repression of genes encoding enzymes required for aerobic respiration and activation of a few genes required for anaerobic metabolism.

Upon shift from aerobic to anaerobic conditions, an Hfq-dependent sRNA, FnrS, is synthesized. Transcription of the *fnrS* gene is activated by FNR and ArcA and to a lesser extent by CRP (Boysen et al., 2010; Durand and Storz, 2010). This results in the repression of a number of metabolic enzymes that are dispensable during anaerobic conditions. Single stranded regions from both the 5'-end and the central region of FnrS sRNA are responsible for base pairing with their respective target mRNAs. For instance, the central region is important for regulation of *maeA* and *gpmA*, whereas the 5'-end of FnrS is required to repress *sodB*, *folE*, and *folX* mRNA translation (Durand and Storz, 2010).

In *Neisseria meningitidis*, another FNR activated sRNA, AniS, has been reported during anaerobiosis (Fantappiè et al., 2011). Whole-genome expression analyses led to identification of NMB0214 (encoding PrlC oligopeptidase) and NMB1468 (encoding a lipoprotein) mRNA as putative targets for the AniS *trans*-antisense interaction. In contrast to the role Hfq plays in the function of FnrS, AniS is reported to be less stable in an Hfq+ background, although Hfq has been suggested to promote the base pairing interaction of AniS to its targets (Fantappiè et al., 2011).

PhrS RNA is another regulatory RNA that is induced to thrive under anaerobiosis in *P. aeruginosa* (Sonnleitner et al., 2011). It is



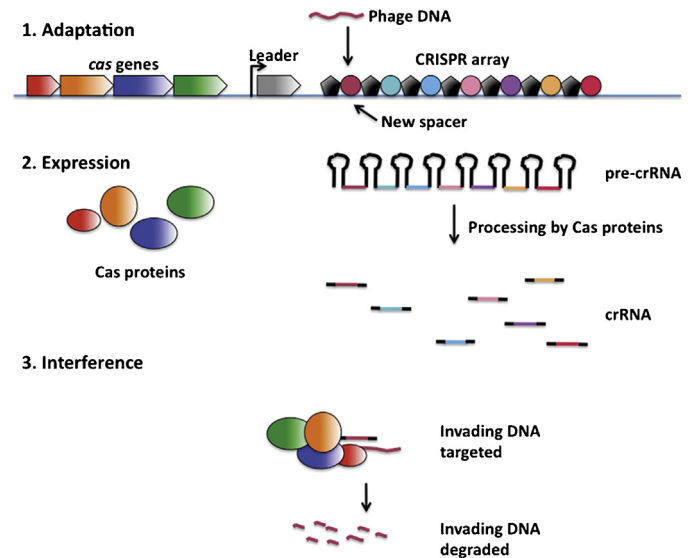
one of the rare examples of a small regulatory RNA that also encodes a peptide (~37 amino acids). Transcription of the *phrS* RNA gene is activated by the oxygen responsive regulator ANR, a homolog of the FNR protein of *E. coli*, which activates a vast network of anaerobic/hypoxic functions when bacteria are exposed to anaerobiosis. PhrS can base pair with the 5'-leader sequence of the transcript that contains two ORFs: the upstream open reading frame (*uof*) and the ORF encoding PqsR (Sonnleitner et al., 2011). Synthesis of the small uncharacterized peptide *uof* and the transcriptional regulator protein PqsR is coupled and structurally repressed during aerobic bacterial growth. The binding site for PhrS regulatory RNA is located about 170 nucleotides upstream of the *uof* translation start codon. When oxygen is scarce, this interaction results in the structural rearrangement of the *uof* region harboring the RBS in the *uof-pqsR* transcript and activates *uof* mRNA translation (Fig. 2A). Elongation of *uof* translation further unwinds the *uof-pqsR* transcript and permits translation of the PqsR protein. The transcriptional regulator PqsR can interact with the *Pseudomonas* quinolone signal (PQS) or PQS precursor (2-heptyl-3-hydroxy-4-quinolone) and drastically increase transcription of the *phnAB* and *pqsABCDE* operons (except *pqsE*), which encode gene products that are essential for PQS synthesis (Déziel et al., 2004; Gallagher et al., 2002). PqsR also is required for transcription of the *phzA1-G1* operon and the *phzS* and *phzM* genes, which are involved in *P. aeruginosa* virulence factor pyocyanin (PYO) biosynthesis (Déziel et al., 2005).

Hence, PhrS-mediated activation of *pqsR* translation leads to increased levels of PQS and PYO. Translational activation of *pqsR* via PhrS is regarded as another mechanism to anticipate stress conditions using regulatory RNA because enzymatic conversion of 2-heptyl-3-hydroxy-4-quinolone to PQS is diminished under limited oxygen conditions (Toyofuku et al., 2008). *P. aeruginosa* can form biofilms that contain mushroom-shaped multicellular structures consisting of stalks and cap-forming sub-populations during anaerobic conditions (Yang et al., 2009). Although cap-forming cells do not express quorum sensing genes and have a lower cell density as compared to microcolonies forming stalk cells, biofilm formation of *P. aeruginosa* under anaerobic condition is enhanced. This increased in quorum sensing is mediated by increased PQS, which triggered by PhrS. Hence PQS enhances the release extracellular DNA from stalk cells stimulating the formation of the biofilm matrix component of the cap-forming sub-population (which increases the amount of extracellular DNA for Biofilm production) (Yang et al., 2009).

### CRISPR: a new mechanism for stress management and adaptive immune response

Bacterial cells endure a never-ending onslaught of virus, phage, and plasmid invasions. For survival, prokaryotes deploy various strategies to deal with stress, such as receptor masking or mutagenesis, blocking of phage DNA injection, restriction/modification, abortive infection, and use of a small RNA-based adaptive immune defense system called clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins (CRISPR/Cas) (Barrangou and Horvath, 2012; Makarova et al., 2011; Westra et al., 2012). The CRISPR/Cas system maintains a genetic record of prior encounters with foreign transgressors and can target invading viruses, phages, or plasmids in three stages: (1) adaptation, (2) expression, and (3) interference (Fig. 4) (Datsenko et al., 2012; Richter et al., 2012). There are three major CRISPR/Cas system types (I, II, and III), which are characterized by the presence of their signature protein (Makarova et al., 2011).

The genomic region containing CRISPR is referred to as the CRISPR locus or CRISPR array (Deveau et al., 2010). The CRISPR locus harbors up to several hundred spacer sequences (~26–72



**Fig. 4.** Overall arrangement and processing of CRISPR RNAs. CRISPR arrays consist of DNA repeats (black pentagons) separated by unique spacers (round shapes with different colors). *Cas* genes, which encode proteins that function in CRISPR RNA processing and/or silencing of invading DNA, are usually located nearby. During adaptation, a short fragment of invading DNA is integrated into the CRISPR locus. The invading DNA is typically incorporated into the leader end of the CRISPR array and the repeat is duplicated. During the expression stage, CRISPR is transcribed into a primary transcript (pre-crRNA) and further processed into crRNAs. During the interference stage, these crRNAs together with Cas proteins target invading RNAs or DNAs and lead to degradation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

nucleotides) flanked by repeats from 21 to 47 nucleotides in length (Godde and Bickerton, 2006; Grissa et al., 2007; Tang et al., 2002). During the adaptation stage, a short segment of DNA from an invading virus, phage, or plasmid (known as the protospacer) is integrated preferentially at the leader end of the CRISPR locus and is accompanied by duplication of the spacer sequence to maintain the repeat-spacer-repeat architecture. At the expression stage, CRISPR is transcribed into primary transcripts (pre-crRNA) and further processed into short CRISPR-derived RNAs (crRNAs) via endonucleolytic cleavage. This system is similar to the RNA interference (RNAi) machinery of eukaryotes, although they are not homologous. For both CRISPR-crRNA and RNAi, precursor RNA transcripts are processed into short guide RNAs by endonuclease-mediated cleavage (Koonin and Makarova, 2009; Marraffini and Sontheimer, 2010). In *Streptococcus pyogenes*, an RNase III endonuclease, which is a functional homolog of the eukaryotic RNAi processing enzyme Dicer, was shown to be involved in maturation of pre-crRNA transcripts (Deltcheva et al., 2011).

At the interference stage, crRNAs associate with Cas proteins to form the CRISPR ribonucleoprotein (crRNP) complex that, based on complementarity, can recognize foreign nucleic acids (Wiedenheft et al., 2012). Formation of duplexes between a cognate protospacer sequence of the crRNA and the foreign DNA (or RNA) guide crRNP-associated endonucleases to cleave the invading DNA (or RNA) targets (Fig. 4) (Barrangou and Horvath, 2012; Makarova et al., 2011).

While the CRISPR/Cas system may be constitutively expressed in archaea (Hale et al., 2008; Tang et al., 2002), CRISPR/Cas expression in eubacteria seems to depend on signals. For instance, expression of CRISPR/Cas transcripts in *Thermus thermophilus* is increased following phage infection (Agari et al., 2010). In *E. coli*, H-NS protein has been demonstrated to repress the transcription of *casA* and the CRISPR I promoter (Pul et al., 2010), whereas transcription factor LeuO can displace H-NS and activate transcription of *cas* genes (Westra et al., 2010). The BaeSR two-component regulatory system,

which responds to envelope stress, was also recently demonstrated to be instrumental in activating CRISPR defense via release of H-NS-mediated *cas* repression (Perez-Rodriguez et al., 2011). Although the detailed mechanisms underlying the different stress responses and activation of the CRISPR/Cas system have yet to be uncovered, the aforementioned findings indicate an interconnection between bacterial stress and an RNA-based adaptive immune defense system. A number of questions remain: Is it possible that envelope stress forms an alliance with bacterial innate defense by buying more time by activating CRISPR defense? Does quorum sensing play a role in this? It remains to be seen whether other stress conditions or even sRNAs that are induced under certain stress conditions can trigger the CRISPR/Cas machinery.

### Future perspectives/concluding remarks

Recent advancements in RNA research have provided new insights about how RNAs are utilized to regulate a plethora of cellular activities, including different stress responses in bacterial pathogens. RNA thermometers, riboswitches, *cis/trans*-encoded sRNAs, CRISPR, and other small bacterial sRNAs regulate different stress responses by switching between alternate structural conformations; by base pairing to target RNAs that modulate translational activity and stability of mRNAs or entire operons; by selective targeting of alien nucleic acids for degradation; or by binding to regulatory proteins and controlling their activity.

It is noteworthy to highlight the advantages of utilizing sRNA-mediated stress responses in comparison to regulation by protein transcriptional factors. Bacterial sRNAs are small and usually are not translated into proteins or peptides; therefore they require less energy and time for their synthesis and turnover. Hence, they are more economical in comparison to transcriptional regulator proteins that have to undergo the extra step of translation. In addition, many sRNAs act at the post-transcriptional level, which ensures a rapid response to stress signals. In addition, the versatility of sRNAs provides a new dimension in terms of how the target gene can be regulated as compared to protein transcriptional regulators, thus contributing to an additional layer of regulation. Indeed, the combination of regulation using both sRNA and transcriptional factors has been reported to alter the target gene expression dynamics and to minimize the leakiness in expression (Beisel and Storz, 2010; Levine et al., 2007). Unique kinetic regulatory properties displayed by sRNA-mediated regulation also are advantageous compared to transcription factors, especially when the triggering signals are persistent, as in the case of stress signals (Levine and Hwa, 2008; Levine et al., 2007).

Depending on the type of stress signals, some essential proteins for virulence are also repressed, as demonstrated by VrrA and RyhB. The topics discussed herein could lead to alternative avenues for the development of novel means of bacterial diagnosis, treatment, or even sRNA-based technologies. For example, the riboswitch was exploited as a target for drug analogs to reduce pathogenesis of *S. aureus* in mice (Mulhbacher et al., 2010). In addition, CRISPR is currently being applied in gene targeting and chromosome engineering. It was recently reported that the Cas9 in complex with guide synthetic sRNA could specifically target and cleave DNA sequences of interest (Cong et al., 2013; Jinek et al., 2012; Mali et al., 2013). In line with transcription activator-like effector nuclease (TALEN) (Bedell et al., 2012; Miller et al., 2011; Wood et al., 2011) and zinc finger nuclease (ZFN) (Carroll, 2011; Miller et al., 2007; Urnov et al., 2010; Wood et al., 2011) approaches, application of the CRISPR/Cas system in mammalian gene targeting could open a new era of transgene and gene knock-out technologies. Undeniably, these powerful technologies could lead to exciting novel findings

while serving as an alternative to investigate gene functions and genetic diseases in the future.

Collectively, with the fast pace of high throughput technology coupled with advancements in bioinformatics, additional sRNAs involved in regulation of bacterial stress conditions are expected to keep emerging. The next major challenge lies in better understanding the role of sRNAs in bacterial biogenesis and pathogenesis. Such knowledge could provide important insights for potential therapeutic application of sRNA as novel diagnostic markers and drug targets.

### Conflict of interest

The authors declare no conflict of interest.

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