Most bacteria encode a large repertoire of RNA-based regulatory mechanisms. Recent discoveries have revealed that the expression of many genes is controlled by a plethora of base-pairing noncoding small regulatory RNAs (sRNAs), regulatory RNA-binding proteins and RNA-degrading enzymes. Some of these RNA-based regulated processes respond to stress conditions and are involved in the maintenance of cellular homeostasis. They achieve it by either direct posttranscriptional repression of several mRNAs, including blocking access to ribosome and/or directing them to RNA degradation when the synthesis of their cognate proteins is unwanted, or by enhanced translation of some key stress-regulated transcriptional factors. Noncoding RNAs that regulate the gene expression by binding to regulatory proteins/transcriptional factors often act negatively by sequestration, preventing target recognition. Expression of many sRNAs is positively regulated by stress-responsive sigma factors like RpoE and RpoS, and two-component systems like PhoP/Q, Cpx and Rcs. Some of these regulatory RNAs act via a feedback mechanism on their own regulators, which is best reflected by recent discoveries, concerning the regulation of cell membrane composition by sRNAs in *Escherichia coli* and *Salmonella*, which are highlighted here.

**Introduction**

Small regulatory RNAs (sRNAs) are ubiquitous multipurpose regulators present in all domains of life. They act via complex interactions with DNA/chromatin, proteins, other RNA molecules and metabolites [1]. In bacteria, generally their transcript size varies from 50 to 300 nucleotides. Base-pairing sRNAs can be either cis-acting or trans-acting, depending on their genomic location and with respect to their target mRNAs [2,3]. Many of these sRNAs function by imperfect base pairing with multiple mRNA targets and often require the help of the RNA chaperone Hfq [2]. Base-pairing sRNAs act as major posttranscriptional regulators that can either enhance or repress mRNA decay and/or translation by binding to the 5' untranslated region (UTR) of mRNA targets [2,3]. However, some sRNAs function by the interaction with regulatory proteins, often acting by sequestration. Initially, sRNAs were identified by the analysis of transcription from intergenic regions [4]. Extensive RNA-seq analysis of transcriptome of various regulators, identification of RNAs by ligating Hfq-bound sRNAs and sequencing sRNA–RNA interactions (RIL-seq) or by UV-cross-linking sRNA–target RNA duplexes to RNase E (CLASH) or immunoprecipitations with Hfq and ProQ have vastly increased the repertoire of sRNAs and their targets [5–8]. These studies have revealed that sRNAs can originate from various genomic regions, including antisense to coding regions [6,8], 3' UTRs [9–12], 5' UTRs [13] and even coding regions [6] (Figure 1). Even in bacteria that lack Hfq, like *Mycobacterium tuberculosis*, the non-coding RNA content can be up to 58% of the total non-rRNA in the stationary phase [14]. Specific sRNAs control the expression of sigma factors like the stationary-phase sigma factor RpoS, the envelope stress-responsive sigma factor RpoE and the activity of house-keeping sigma factor RpoD [2,3,10]. The sRNA-mediated control has also been described for several regulatory factors like FlhDC (motility/flagellar expression), Lrp (amino acid biosynthesis), CRP (catabolite repression) and SoxS.
Thus, sRNAs are deployed by each of the major stress-responsive pathways, controlling the expression of crucial regulatory molecules that can rapidly alter gene expression profiles, allowing fast adjustment to different growth conditions.

Transcription of most trans-acting sRNAs is controlled by transcriptional factors that include global regulators, two-component systems (TCSs) and specific stress-responsive sigma factors [17,18]. One of the well-studied cases is the regulation of envelope stress response by the RpoE sigma factor, the Cpx TCS and regulatory sRNAs that
are members of their regulons [12,18]. RpoE is specifically induced in response to outer membrane protein (OMP) misfolding, defects in the lipopolysaccharide (LPS) composition and an imbalance between phospholipids and LPSs [10]. Interestingly, RpoS, RpoN, RpoD, RpoE sigma factors and TCSs, like Rcs and QseE/F, positively regulate transcription of the \( rpoE \) gene in response to specific stress conditions (Figure 2) [13]. However, the RpoE availability/activity is also subjected to a negative regulation by the RseA anti-sigma factor and a feedback control by RpoE-regulated sRNAs [10,19,20]. As several sRNAs, belonging to stress-responsive regulons, also act in a negative feedback mechanism, this suggests functional overlaps and co-integration of signals to achieve homeostasis. Consistent with integration of diverse signals, the expression of many stress-responsive regulators and sigma factors are regulated by sRNAs (Figure 2) [17,18]. In this review, we address how major stress-responsive regulatory systems engage sRNAs to regulate the envelope stress response and co-ordinate the gene expression upon other specific stresses.

**Regulation of the RpoE-dependent extracytoplasmic stress response by sRNAs**

The cell envelope of Gram-negative bacteria, such as *Escherichia coli* or *Salmonella*, is composed of an asymmetric outer membrane (OM) that is separated from the inner membrane (IM) by the periplasmic space containing the peptidoglycan. The content and assembly of OM components, such as OMPs, lipoproteins and LPSs, are tightly regulated. This involves control mechanisms exerted by sRNAs and cell envelope stress-responsive pathways that detect and respond to any dysfunction in the assembly or an imbalance in different components of the cell envelope. Hence, defects in either OMP assembly or LPS synthesis or exposure to stress conditions induce cellular responses that are transcriptionally regulated by the alternative sigma factor RpoE and TCSs like CpxR/A, Rcs, ...

**Figure 2. Stress-responsive sigma factors regulated sRNAs.**

The network of the repressing arm of RpoE/Cpx systems exerted via its sRNA regulon members and the hub of some of sRNAs that either regulate the RpoS expression or are regulated by RpoS. sRNAs are shown in red color and pink color refers to two-component systems. The repressive arm of RpoE by RybB, MicA and SlaA (MicL) that repress OMP synthesis is presented. Suppression of Spk synthesis by the CpxQ sRNA is also shown. RpoS-regulated sRNAs are indicated and their major targets are pointed.
PhoP/Q, EnvZ/OmpR and BasS/R [21,22]. Some of these regulons often encode sRNAs that serve to provide a rapid response and can either amplify the signal or act by a negative feedback. Furthermore, some response regulators are subject to a posttranscriptional regulation by sRNAs, as is the case with the RpoE-regulated MicA-mediated repression of phoP mRNA synthesis linking RpoE and the PhoP/Q TCS to control OM constituents: OMPs and LPSs [23]. MicA is a major sRNA initially identified as the posttranscriptional repressor of ompA mRNA synthesis [24]. Similarly, two OmpR-regulated sRNAs, OmrA and OmrB, regulate the expression of certain OMPs like OmpT and also provide a negative feedback to the OmpR regulator [25].

**RpoE-regulated MicA and RybB control of OMP synthesis**

The RpoE sigma factor is specialized in its function dedicated to the maintenance of OM homeostasis. The coding arm of the RpoE regulon includes factors that promote OMP synthesis/folding, LPS remodeling and its assembly/transport [18,26]. Defects in OMP or LPS biosynthesis strongly induce the RpoE activity, and here the noncoding arm of the RpoE regulon counteracts deleterious effects of the RpoE expression [13,20–22]. Previously, it was noted that overexpression of RpoE or activation of the RpoE pathway in the absence of the anti-sigma factor RseA leads to repression of OMP synthesis [26,27]. This is now best explained by discoveries of three well-conserved RpoE-transcribed Hfq-binding sRNAs rybB, micA and slrA (micL), which repress the synthesis of abundant OMPs (RybB- and MicA-dependent) and the most abundant lipoprotein Lpp [SlrA (MicL)-dependent] (Figure 2). RybB and MicA together target >30 mRNAs of *E. coli* [20], providing a repressive arm that alleviates stress and mediating interconnection with other envelope stress response networks. MicA and RybB target all *omp* mRNAs and some non-*omp* targets. Thus, MicA represses the synthesis of major OMPs by targeting mRNAs of *ompA*, *ompX* and *lamb* and non-OMP targets like *phoP*, *lpxT* and *htrG* [20,23]. RybB has even more in vivo *omp* mRNA targets and non-*omp* targets that include *waaR*, *htrG*, *fadL* and *rbsK/B*, which are repressed by RybB (Figure 2) [20,22,28]. RybB and MicA bind their target mRNAs using a seed region of 6–8 contiguous bases for base pairing [2,20,29]. This base pairing, involving the seed region of sRNA, can occur close to the ribosome-binding site or within so-called 5-codon region located close to the 5′ end of the coding sequence or located deep within the coding sequence [30]. The interaction within the coding sequence between sRNA and target mRNA can cause mRNA decay without ribosome binding [30]. Overall, the main function of either MicA or RybB sRNA is to protect the cell from the loss of viability when the RpoE activity is inadequate.

**The negative feedback control of RpoE by the RpoE-regulated SlrA (MicL) and repression of Lpp synthesis**

SlrA (MicL) was identified independently by two groups, during characterization of suppressors of Δ*lapB* mutants that overproduce LPSs, called SlrA [10], and during examination of RpoE-regulated sRNAs, called MicL [11]. The lapB gene is essential and its absence leads to increased synthesis of LPS at the expense of phospholipids, aggregation of proteins involved in LPS biosynthesis and decoupling of LPS synthesis with LPS transport [10]. Consequently, Δ*lapB* exhibits hyper-elevated levels of RpoE and Cpx regulons. The main function of SlrA (MicL) was shown to be the Hfq-dependent inhibition of *lpp* mRNA and repression of the RpoE activity. In support of these results, overproduction of SlrA mimics Δ*lpp* phenotype [10]. Since Lpp contains three acyl chains, reduction in Lpp synthesis by SlrA can liberate pools of phospholipids that can overcome toxicity due to the excess of LPS and depletion of the common precursor for phospholipids. Interestingly, SlrA (MicL) is encoded within the *cutC* gene and is transcribed from its own RpoE-regulated promoter [10,11]. This sRNA is synthetized as a precursor of 308 nt and is processed to a mature 80-nt RNA located within the 3′ UTR of the *cutC* gene (Figure 1A). Importantly, overexpression of *slrA* strongly represses elevated toxic levels of the *rpoE* regulon in Δ*lapB* defects [10]. This negative feedback control of the RpoE expression by increased synthesis of SlrA provides a robust mechanism of maintaining cellular homeostasis by the sRNA arm of RpoE. Indeed, recent global studies have revealed mRNAs of *rpoE*, *acpP*, *fadE*, *maxF* and *fixA* as additional base-pairing targets of SlrA (MicL) [6]. If the translation of the *rpoE* mRNA is blocked by SlrA, this may explain the lack of any significant increase in the RpoE amount under conditions that increase the RpoE transcriptional activity. For instance, in *E. coli*, severe defects in LPSs known to trigger RpoE induction have unaltered total amount of RpoE [31], since under such conditions transcription of the *slrA* gene will also be induced counteracting the increase in synthesis of RpoE. However, it is pertinent to point out that burst in the RpoE activity when LPS is defective is due to the liberation of RpoE from RseA [31]. Taken together, three RpoE-regulated sRNAs (RybB, MicA and
SlrA) provide a negative feedback arm, as in their excess the induction of RpoE stress response is dampened by reducing unwanted buildup of OMPs under stress conditions and this could allow redirection of RpoE-regulated chaperones to mediate protein folding in the periplasm.

The sRNA-dependent remodeling of LPS and integrated control by RpoE and PhoP/Q
Under RpoE-inducing conditions, E. coli remodels its LPS synthesis that is controlled by RpoE-regulated sRNAs, particularly RybB. Upon the RpoE induction, the RybB sRNA represses the synthesis of WaaR glycosyltransferase, causing truncation in the outer core of LPSs and concomitant incorporation of a third Kdo (WaaZ-dependent) and rhamnose in the inner core [22]. Interestingly, under such conditions, the waaZ mRNA accumulation also increases, contributing to the synthesis of LPS with a third Kdo [22]. This mode of LPS remodeling could be adaptive in function, as the non-essential waaZ gene becomes essential for the bacterial growth when the LPS is tetraacylated and for interaction with the host, since such an LPS cannot ligate O-antigen due to loss of the attachment site [22]. This drastic shift causing the accumulation of a rare form of LPS is accompanied by a nonstoichiometric modification of the second Kdo by phosphoethanolamine (P-EtN). The transfer of P-EtN to the second Kdo is important for resistance to cationic antibiotics like polymyxin B and is mediated by the product of the RpoE-regulated eptB gene [32]. The eptB mRNA synthesis is usually repressed by the PhoP/Q-regulated MgrR and by the ArcZ sRNAs [32]. However, this silencing by MgrR and ArcZ is overcome when RpoE is induced. MicA contributes to remodeling of LPSs by inhibiting phoP and lpxT synthesis at the posttranscriptional level by direct base pairing with these target mRNAs [20,23]. The PhoP/Q TCS regulates LPS nonstoichiometric alterations and is required for transcription of the mgrR sRNA. The MicA-directed repression of LpxT is interesting, since LpxT mediates the phosphorylation of lipid A that causes an increase in the negative charge of LPS. The GcvB sRNA also negatively regulates the phoPQ expression that is independent of MicA [33]. Interestingly, SoxS, a global regulator of the oxidative stress response, is a positive regulator of transcription of the waaY gene, whose product phosphorylates heptose II and is itself subjected to a negative regulation by the MgrR sRNA [16]. Another sRNA that regulates LPS modification is McF, since base pairing of McF within the coding sequence of the lpxR mRNA decreases its stability by rendering it susceptible to degradation by RNase E [34]. LpxR is a lipid A deacylase and this modification occurs after LPS translocation. Hence, the regulation of LpxR by McF contributes to the LPS modification event that occurs in the OM, expanding the role of sRNAs at various steps of the LPS biosynthesis [18]. Such controls exerted by sRNAs on regulators of the stress response that at the same time regulate LPS structural alterations demonstrate rewiring of networks to cope with envelope stress.

Regulation of the envelope stress response by the Cpx TCS and mediation by the CpxQ sRNA
The CpxR/A TCS is one of the typical TCSs with the IM-located CpxA acting as a histidine kinase and CpxR being the DNA-binding response regulator. The Cpx system responds to protein folding defects in the periplasm and defects in peptidoglycan, and is induced by variety of stresses that include high pH, hyperosmolarity and alterations in the IM lipid composition, and when LPS is composed of either Kdo2-lipid IVα or only free lipid IVα derivatives or in ΔlapB mutants [10,21,35]. The activity of the Cpx system is further fine-tuned by PrpA (serine/threonine phosphatase) that modulates the phosphorylation status of CpxR [36]. Elucidation of RpoE and Cpx regulon members revealed overlap in the RpoE and Cpx pathways, although certain signals are specific for each system [26,35]. The Cpx system is generally activated upon IM perturbations, while RpoE strongly responds to OM signals. The Cpx activity is regulated by the negative feedback mediated by a periplasmically located CpxP protein [35]. Transcription of the cpxP gene is strongly activated upon the induction of the Cpx pathway. As in the case of RpoE, the sRNA-mediated regulatory control has been discovered, which plays an important role in regulating the Cpx TCS and in interconnections with other envelope stress systems. Importantly, it was shown that the transcript of the cpxP gene includes a novel sRNA cpxQ, located within its 3’ UTR [12]. This sRNA uses the same promoter as the cpxP gene and is generated by the RNase E-mediated processing of the 3’ end of cpxP mRNA (Figure 1B). CpxQ in conjunction with Hfq represses the synthesis of NhaB, Skp, Agp and FimA [12]. NhaB acts as a sodium–proton antiporter and its repression by CpxQ can counteract the loss of proton motive force (PMF) during the IM stress and can provide resistance to dissipation of membrane potential. Consistent with these findings, the loss of PMF is a potent inducer of the Cpx TCS and can thus provide a basis for
maintaining homeostasis during envelope stress [12]. Repression of Skp synthesis, a well-known OMP chaperone [37], by CpxQ is another mechanism of counteracting the induction of Skp synthesis upon the RpoE induction that can balance RpoE and Cpx systems. Indeed, in E. coli, it was shown that CpxQ combats toxicity in the IM by down-regulating the synthesis of Skp, preventing insertion of OMPs into the IM by Skp [38]. Thus, the function of cpxQ along with the Cpx system caters to the need of protection against IM damage.

Interestingly, CpxR positively regulates synthesis of OmrA, OmrB, RprA and MicF sRNAs, while repressing the synthesis of CyaR. Targets of OmrA/B sRNAs include mRNAs of several OMP-encoding genes like fecA, ompT, cirA and fepA [25]. In this process, OmrA/B further provides a negative feedback loop to the EnvZ/OmpR system [25]. MicF has been well studied for its negative regulation of OmpF and, in these pathways, the EnvZ/OmpR TCS cross-talks with the Cpx system via RpoE/Cpx-regulated EcFM (MzrA) [26,35]. Since CpxR positively regulates the synthesis of rprA sRNA, it provides a link for Rcs, RpoS and Cpx pathways in the control of stress response [13,35]. The RprA sRNA positively regulates the rpoS expression and is a member of the Rcs regulon. The Rcs system and RpoS further regulate transcription of the rpoE gene in response to LPS defects and entry into the stationary phase by controlling the expression of specific promoters of the rpoE gene [13]. Thus, these noncoding RNAs, sigma factors and TCSs co-regulate the envelope stress response.

### The RpoS-regulated stationary-phase response and a role of sRNAs

The RpoS sigma factor is a global regulator of stress response, transcribing nearly 10% of genes, and acts as a major hub for sRNAs, modulating the expression of nearly 23% sRNAs in Salmonella [39]. RpoS regulon members exhibit overlaps with other stress-responsive controlling sigma factors and TCSs (Figure 2). The expression of the rpoS gene is positively regulated by DsrA, RprA and ArcZ and negatively by OxyS sRNAs [40]. Positively regulating base-pairing sRNAs activate the expression of RpoS by binding to the 5′ UTR of rpoS mRNA in an Hfq-dependent manner by releasing the inhibitory secondary structure and enhancing translation [40]. The RprA expression is activated under acid stress conditions, induction of the Rcs system and exposure to chemical stresses like the presence of succinic acid, acetate and itaconic acid [41]. ArcZ overexpression results in differential expression of over 750 genes and loss of motility [42]. The ArcZ sRNA serves as a connector between the general stress response regulated by RpoS and the specific response mediated by the ArcA/B TCS that responds to a redox state of the respiratory chain. RpoS is directly involved in the transcriptional activation of the rpoE gene via the recognition of the rpoEP4 promoter, which is one of its most active promoters [13]. RpoS directly regulates transcription of gadY, omrA, sdsR, sdiN and srl sRNAs [17,43,44]. RpoS regulates acid resistance by the induction of transcriptional factors GadE/X/W. While the gadY sRNA positively regulates GadX and GadW [2,3], gadE contains a novel sRNA gadF in its 3′ UTR [6]. Overexpression of gadF sRNA up-regulates acrB and cfa expression. Thus, the GadE transcription factor and GadF sRNA are encoded in the same transcript and co-regulate genes in the same pathway (Figure 1C) [6]. The RpoS-regulated SdsR sRNA controls biofilm formation, down-regulation of an abundant porin OmpD in Salmonella and the expression of mismatch repair protein MuttS [17]. SdsR could play additional roles in stress response with the identification of 20 new down-regulated targets that include HtpG (conserved heat shock protein), global regulators like CRP (catabolite repression), StpA (nucleotide-associated protein) and ToIC (efflux pump protein) [17]. SraL is quite strongly induced in an RpoS-dependent manner, and it targets the tig mRNA in Salmonella [43]. The tig gene encodes the ribosome-associated abundant peptidyl prolyl cis/trans isomerase. Repression of the Tig expression under the stationary phase could be a part of the homeostasis mechanism to reduce protein synthesis. Two small sRNAs, SdsN137 and SdsN178, are transcribed from two RpoS-dependent promoters, but share the same terminator [44]. Overexpression of SdsN137 causes repression of NfsA nitroreductase and hence regulates resistance to nitrofurans [44]. Taken together, RpoS-regulated sRNAs connect various stress regulons like RpoE and RpoH, total protein expression and response to acid resistance under stationary-phase conditions.

### sRNAs that act by interacting with regulatory proteins

Some sRNAs regulate the gene expression by interacting with regulatory proteins, modifying their activity, and generally act via protein sequestration. Two families of such widely conserved sRNAs, represented by the CsrB/RsmZ family and by 6S RNA, have been well characterized. In addition, a new sRNA RhsA interacts with RfaH, and this interaction potentially alters the RfaH conformation, rendering it NusG-like, thereby reducing RfaH’s ability to recognize its target operons.
The CsrB family of sRNAs
The CsrB family of sRNAs uses short sequence AUGGA motifs in apical loops of RNA secondary structure to bind multiple copies of carbon storage regulator A (CsrA), leading to its sequestration. CsrA acts as a translational repressor of several mRNAs and regulates a plethora of phenotypes, including carbon storage, secondary metabolism, motility, biofilm formation, peptide uptake, cyclic di-GMP and (p)ppGpp synthetases, quorum sensing and the expression of virulence genes [5,45]. CsrB-like sRNAs contain as many as 18 CsrA-binding motifs. When these sRNAs are in high amounts, CsrA binds to them and hence is not available to bind its target mRNAs [5]. This sequestration causes up-regulation of CsrA targets conferring pleiotropic phenotypes in biofilm, gluconogenesis, capsule formation and impacting virulence [5]. In E. coli, overexpression of the csrB sRNA mimics a csrA mutation, leading to increased glycogen synthesis [5,45]. The synthesis of CsrB and CsrC is regulated by the BarA/UvrY TCS, and this regulatory cascade is conserved in several bacteria. Furthermore, the McaS sRNA can sequester CsrA, causing repression of PGA exopolysaccharide synthesis [46]. As CsrA and CsrB-like sRNAs are conserved, they serve as a model for study of such sRNA interactions with regulatory proteins.

The regulatory role of 6S RNA
The abundant 6S RNA is conserved in Gram-negative and Gram-positive bacteria and functions by binding at the active site of σ70-RNA polymerase by virtue of its double-stranded RNA hairpin structure that mimics DNA in an open promoter complex [47]. 6S RNA accumulates to high amounts in the stationary phase, sequesters σ70-RNAP and favors transcription by the RpoS-containing RNA polymerase. Recovery from the 6S RNA inhibition, upon transition from the stationary phase into the exponential phase, occurs by the release of 6S RNA from the inhibited RNAP complex involving synthesis of a short transcript of ∼14 nt (pRNA), wherein 6S RNA acts as a template that requires a high NTP concentration. The formation of the pRNA–6S RNA complex induces a structural rearrangement that triggers release of 6S RNA, which is subsequently removed by degradation [48].

The RirA sRNA and its interaction with RfaH
RfaH is a paralog of the NusG family of transcription factors. However, RfaH is unique in its specificity for recognition of only those operons that contain a short 8-nt conserved sequence called the ops pause site in the 5' UTR. RfaH transcribes genes that play important functions in virulence such as synthesis of the LPS core, O-antigen, hemolysin and capsule, and for conjugation [13,49]. The RirA sRNA (RfaH-interacting RNA) was identified while analyzing signals that induce transcription of the rpoE gene. Overexpression of the 73-nt RirA sRNA, located in the 5' UTR of the waaQ operon, increases transcription from the rpoE promoter that specifically responds to LPS defects [13]. RirA shares its 5' end with the waaQ mRNA and contains the conserved ops and JUMPstart sites that are recognized by RfaH (Figure 1D). RirA binds RfaH in the presence of RNAP, and this binding requires the presence of the ops site. RirA overexpression abrogates the synthesis of O-antigen, and causes reduction in LPS amounts and truncation in the LPS core, thus mimicking the ΔrfaH phenotype (Figure 1D) [13]. Thus, RirA could either sequester RfaH or act by inducing conformational change in RfaH that renders it into a NusG-like conformation. Free RfaH without interaction with the ops site exists in an inactive closed conformation with its C-terminal domain (CTD) folded in an α-helical hairpin tightly packed against its N-terminal domain (NTD). In this state, the NTD is not accessible for DNA–RNAP interaction. However, when RfaH binds to the ops site, there is a conformational change causing the opening of CTD and NTD, in which RfaH CTD folds into a β-barrel (like CTD of NusG), allowing recruitment of S10 ribosomal protein to couple transcription with translation and also enhance transcriptional elongation [49].

Conclusions and perspectives
Advances in identifying and characterization of bacterial sRNAs have led to our understanding that RNA regulators are ubiquitous. The majority of sRNAs act in trans and can be encoded within almost every possible genomic element. sRNAs, in general, serve as diverse regulators that affect every aspect of bacterial physiology. Many sRNAs have specific functions in relieving OM or IM stress, control of LPS composition and heterogeneity, and regulation of entry into the stationary phase, and in pathogenic bacteria sRNAs could have a specific role during contact with the host. New technologies, identifying sRNA–mRNA interactions like RIL-seq and RNase E-CLASH, have revealed many new potential targets of already known sRNAs and also identified
additional potential sRNAs [6,8]. Recent studies continue to discover new sRNAs, as shown by the transcriptome analysis of *Salmonella* using starvation-stress-responsive conditions that revealed 58 previously unknown sRNAs mostly located in intergenic regions, some of which are conserved in Enterobacteriaceae [50]. Challenges lie ahead in validating these new targets of sRNAs and how these aspects of regulation are integrated into different regulatory circuits. Similarly, future studies are required to understand how a regulatory sRNA like RirA can change the conformation of RfaH that prevents recognition of its target operons that contain the *ops* site and transform it into a general transcription factor like NusG.

**Abbreviations**

CsrA, carbon storage regulator A; CTD, C-terminal domain; IM, inner membrane; LPS, lipopolysaccharide; NTD, N-terminal domain; OM, outer membrane; OMP, outer membrane proteins; P-EtN, phosphoethanolamine; PMF, proton motive force; RpoE, extracytoplasmic function sigma factor; SirA(MicL), suppressor of Lap deficiency and repressor of lpp mRNA; sRNA, small regulatory RNA; TCS, two-component system; UTR, untranslated region.

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**Competing Interests**

The Authors declare that there are no competing interests associated with the manuscript.

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