OPINION

Competing endogenous RNAs: a target-centric view of small RNA regulation in bacteria

Lionello Bossi and Nara Figueroa-Bossi

Abstract | Many bacterial regulatory small RNAs (sRNAs) have several mRNA targets, which places them at the centre of regulatory networks that help bacteria to adapt to environmental changes. However, different mRNA targets of any given sRNA compete with each other for binding to the sRNA; thus, depending on relative abundances and sRNA affinity, competition for regulatory sRNAs can mediate cross-regulation between bacterial mRNAs. This 'target-centric' perspective of sRNA regulation is reminiscent of the competing endogenous RNA (ceRNA) hypothesis, which posits that competition for a limited pool of microRNAs (miRNAs) in higher eukaryotes mediates cross-regulation of mRNAs. In this Opinion article, we discuss evidence that a similar network of RNA crosstalk operates in bacteria, and that this network also includes crosstalk between sRNAs and competition for RNA-binding proteins.

Non-coding small RNAs (sRNAs) have been found in all species of bacteria examined to date. In *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and other species in which the sRNA repertoires have been investigated in detail, the majority of sRNAs are post-transcriptional regulators of major biological processes, including various stress responses, adaptation to nutritional transitions, quorum sensing, biofilm formation, motility and pathogenesis[1–5].

The most common mechanism of regulation by bacterial sRNAs involves the establishment of short, often imperfect base-pair interactions with target mRNAs, thereby forming an sRNA–mRNA duplex that interferes with the binding of the 30S ribosomal subunit to the ribosome binding site (RBS), which is located in the translation initiation region of the mRNA. sRNAs in this class range in size between 50 and 250 nucleotides, and have target sequences that are usually located in 5′ untranslated regions (UTRs) and that can be as short as 6–7 nucleotides. The result of sRNA-mediated interference of ribosomal binding is the inhibition of translation initiation, which can, in turn, stimulate mRNA cleavage by RNase E[6,7] (FIG. 1a) and/or promote premature Rho-dependent transcription termination[8] (FIG. 1b). In Gram-negative bacteria, the activity of most regulatory sRNAs is dependent on their binding to the Hfq homohexamer, which is a ring-shaped protein complex that accelerates sRNA–mRNA annealing[9,10] and protects unpaired sRNA from degradation by RNase E[11]. Occasionally, Hfq can inhibit translation initiation directly by occluding the ribosome binding site[12], in which case sRNAs can have a role in guiding Hfq to the site of interaction[13,14]. In contrast to Gram-negative bacteria, Hfq does not seem to be required for the stability and function of sRNAs in Gram-positive bacteria. Although Gram-positive bacteria encode Hfq-like proteins in their genomes, the role of these proteins is unclear and debated[15–17]. Other mechanisms that have been reported for post-transcriptional regulation by bacterial sRNAs do not always result in the inhibition of translation initiation. In some cases, sRNA base-pairing with an mRNA promotes conformational rearrangements that stimulate mRNA translation (FIG. 1c), whereas in other cases the sRNA sequesters nuclease recognition sites to inhibit degradation and thus stabilize the mRNA[18] (FIG. 1d).

After the sRNA anneals to its target, one of two alternative pathways can occur: a stoichiometric pathway, in which the sRNA is degraded together with the mRNA molecule, or a recycling pathway, in which the sRNA is reused for the regulation of another mRNA molecule[6,19]. The factors that determine the choice between the stoichiometric and recycling pathways are currently unclear, but the finding that the same sRNA can either be degraded or reused depending on the mRNA target[19,20] suggests that the fate of each sRNA is dictated, in some way, by structural features of the sRNA–mRNA duplex. Quantitative analyses suggest that the fate of each sRNA also depends on its abundance relative to that of the target mRNA. Specifically, a 'threshold linear response' is predicted to occur, whereby the sRNA is ineffective until its rate of synthesis exceeds that of the mRNA, at which point the sRNA rapidly, and completely, silences the mRNA[21,22]. Conversely, under conditions in which the accumulation rate of the mRNA is greater than that of the sRNA, the mRNA will sequester or inactivate the sRNA; in other words, the regulator and target will switch roles. A further prediction is that if the sRNA has several targets, an increase in the rate of production of one of these mRNAs will cause sRNA availability for the other targets to decrease below the threshold value and thus cause their deregulation[21,22].

Are these predictions met in living cells? Initial evidence that this is the case came from work on microRNAs (miRNAs), which are ubiquitous 21–23 nucleotide RNAs in animal and plant cells that participate in the silencing of numerous mRNAs (in many cases, an miRNA can recognize hundreds of target mRNAs) by a base-pairing mechanism[23]. Similarly to bacterial sRNAs, miRNAs use a 6–8 nucleotide seed sequence to form base-pair interactions with mRNA targets. A study in 2007 showed that the mRNA targets of miRNAs in animal cells

could be derepressed by expressing RNA sequences that were designed to form base-pair interactions with the seed regions of the miRNAs, thereby competing with the target for miRNA binding[24]. The authors of the study coined the term 'microRNA sponges' to describe these competitive inhibitors of miRNA activity. At about the same time, the first endogenous sponge was identified in *Arabidopsis thaliana*. The sponge, which is encoded in the non-protein coding gene induced by phosphate starvation 1 (*IPS1*), attenuates an miRNA-mediated response to phosphate starvation by mimicking the natural target of the miRNA miR-399 (REF. 25). Subsequently, various natural sponges have been identified in plant and animal cells[26,27]. The findings that miRNAs can be sequestered by sponges that are able to mimic target mRNAs were integrated into a model known as the 'competing endogenous RNA (ceRNA) hypothesis' (BOX 1), which proposes that competition for a shared miRNA regulator establishes hierarchical crosstalk between RNA competitors[28]. Although some reports have questioned the physiological relevance of regulation by ceRNAs[29,30], others have supported such a role; for example, studies that suggested a role for ceRNA pairs in tumour suppression and oncogenesis[31] or that used quantitative modelling to show the potential for regulatory crosstalk between ceRNAs[32–34].

The first description of an active ceRNA pair in bacteria involved competition for the ChiX sRNA in *S. enterica* and *E. coli*[19,20], which was reported in 2009. For five years, competition for ChiX remained an isolated case of ceRNAs in bacteria; however, an increasing number of examples of ceRNAs have recently been reported in *S. enterica*, *E. coli* and *Pseudomonas aeruginosa*, and these ceRNAs have important roles in several biological processes. Some of these examples rely on base-pairing mimicry as the basis for sRNA-mediated mRNA crosstalk, whereas others show interesting variations, such as the use of alternative sets of pairing sequences or the sequestration of an effector protein or cofactor. In contrast to

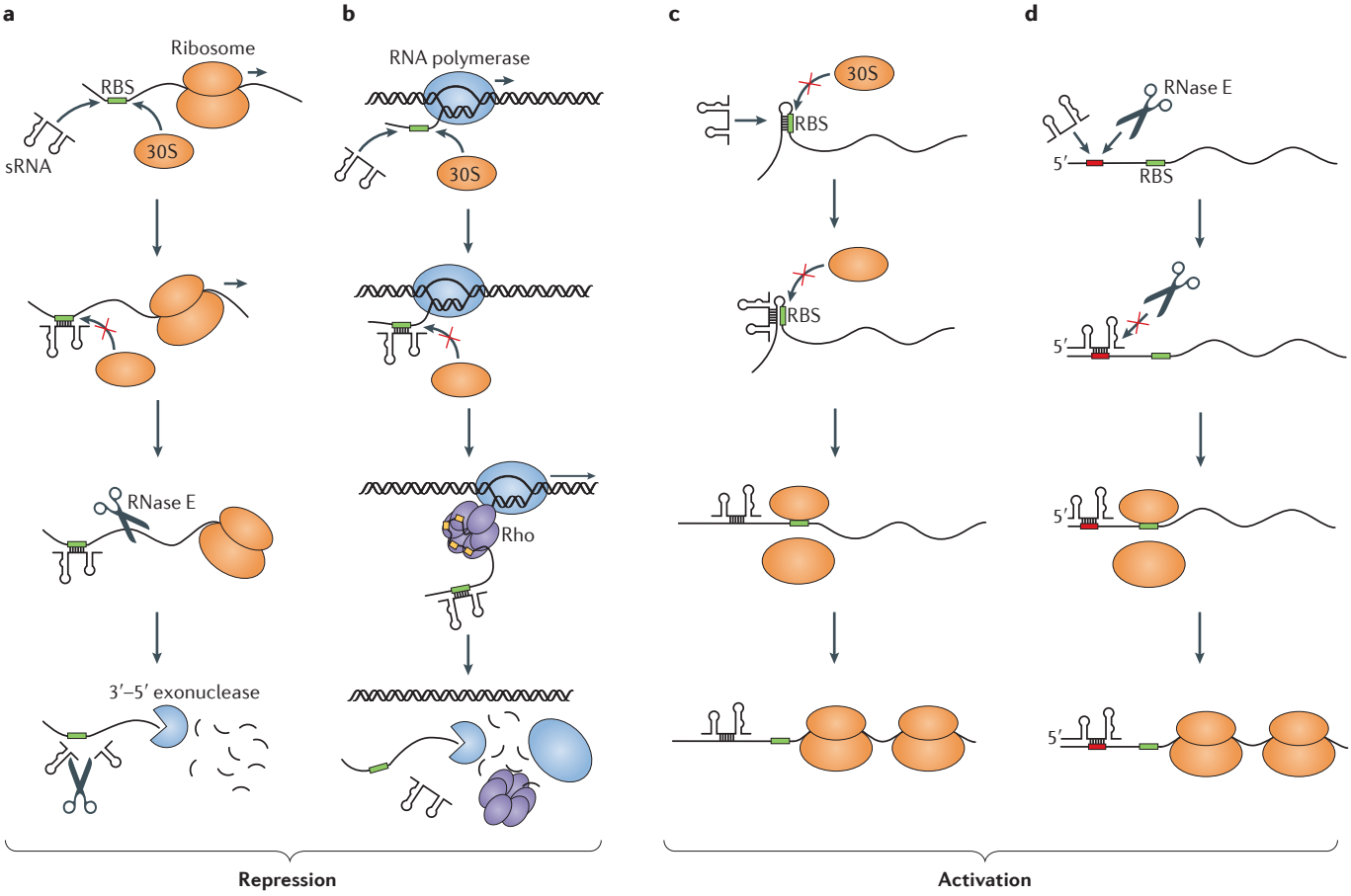


Figure 1 | **Mechanisms of sRNA-mediated regulation in Gram-negative bacteria.** Most small RNAs (sRNAs) associate with the RNA-binding protein Hfq (not shown) and regulate several mRNA targets by forming base-pair interactions with sequences in 5′ untranslated regions (UTRs). In most cases, the target sequence overlaps with the ribosome binding site (RBS), which results in the inhibition of translation initiation through steric hindrance, thereby negatively regulating the target mRNA (part **a**). Furthermore, loss of ribosome protection renders the mRNA susceptible to cleavage by RNase E and subsequent exonucleolytic decay. The sRNA is often degraded at the same time as the mRNA. Alternatively, sRNAs can regulate mRNA targets by binding to the mRNA co-transcriptionally to decouple transcription and translation (part **b**). If a sequence that is recognized by the transcription termination factor Rho is present in the nascent mRNA (yellow rectangles), Rho binding and translocase activity cause premature transcription termination. By this mechanism, the sRNA can silence downstream cistrons in polycistronic transcripts. sRNAs can also be positive regulators of target mRNAs. For example, some mRNAs have secondary structures that sequester the RBS, which limits its accessibility and results in a poor efficiency of translation initiation. In these instances, sRNA pairing with sequences in one arm of the hairpin-like structure can expose the RBS and thus stimulate translation initiation (part **c**). Finally, some mRNAs are inherently unstable, owing to degradation by RNase E. sRNAs can positively regulate these mRNAs by binding to the RNase E entry site, thereby masking the site from RNase E so as to stabilize the mRNA and activate its expression (part **d**).

regulation by eukaryotic ceRNAs, which, in most cases, involves pseudogene transcripts, the emerging picture from studies in bacteria is that bacterial ceRNAs are usually functional mRNAs that are downregulated by an sRNA under certain conditions, but switch to functioning as an sRNA sponge following accumulation in response to environmental cues. In this Opinion article, we discuss the essential features of each of the regulatory systems that have been described for bacterial ceRNAs, including both ceRNAs that compete for a shared sRNA regulator and ceRNAs that compete for a regulatory protein. Finally, we argue that ceRNAs constitute an important new component of the regulatory landscape of bacterial cells.

Competition for a shared RNA regulator

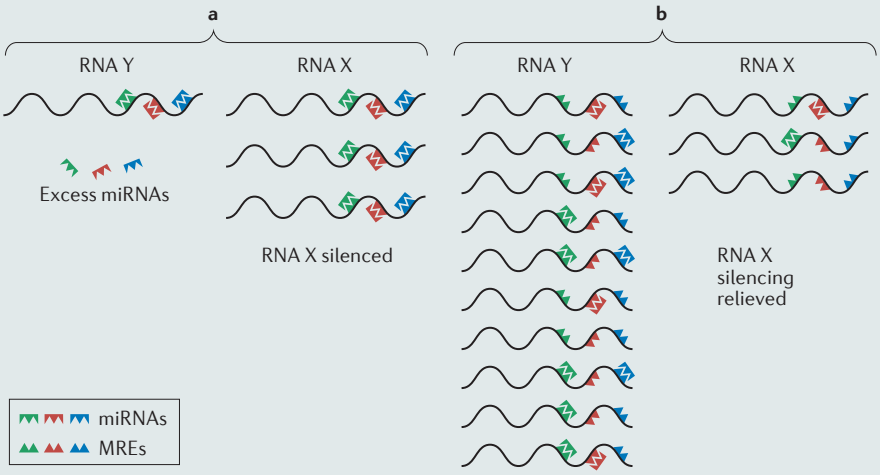
Crosstalk between mRNAs. As the most abundant nitrogen-containing polysaccharide on Earth, chitin is an important source of amino sugars in marine and soil environments, where it is degraded by various chitinase-secreting microorganisms[35]. *S. enterica* and *E. coli* can use the breakdown products of chitin, chitobiose and chitotriose, as sole carbon and nitrogen sources. These chitosugars enter the cell through a dedicated outer membrane porin, chitoporin (ChiP), and are translocated to the cytosol by a mechanism that relies on phosphorylation by the phosphotransferase system that is encoded by the first three genes of the *chbBCARFG* operon[36]. Expression of both *chiP* and the *chb* operon is tightly regulated. Under non-inducing conditions, *chiP* is transcribed at a relatively high basal level but the mRNA is post-transcriptionally silenced by ChiX, which is a constitutively expressed sRNA that inhibits translation by forming base-pair interactions with the ribosome binding site of the *chiP* mRNA. The *chb* operon is repressed at the transcriptional level by the *N*-acetylglucosamine repressor (NagC)[37].

In the presence of chitobiose or chitotriose, transcription of the *chb* operon is activated and, concomitantly, translational silencing of *chiP* is relieved. A *chbB–chbC* intercistronic spacer sequence in the polycistronic *chb* mRNA is recognized as a target by ChiX and elicits the degradation of the sRNA upon pairing with it, thereby depleting the pool of ChiX such that *chiP* is derepressed[19,20] (FIG. 2a). Thus, the *chb* operon encodes a ceRNA that, as an RNA decoy, provides an effective mechanism to couple its own expression with the activation of *chiP* expression. The presence of a ~400 nucleotide RNA that contains the spacer sequence in wild-type cells — and its absence in cells that lack ChiX or that are defective in RNase E activity — suggests that the decoy sequence acts as part of a much longer RNA that undergoes RNase E cleavage upon pairing with ChiX, thereby generating the ~400 nucleotide intermediate[20]. Nonetheless, the *chbB–chbC* intercistronic spacer sequence maintains its capacity to capture ChiX and induce cleavage of the sRNA even when removed from its natural context and expressed ectopically. By comparison, the leader sequence of the *chiP* RNA, which contains the RBS and thus the sequence that pairs with ChiX, is much less effective at causing such cleavage, which is in agreement with the demonstration that ChiX is recycled, rather than degraded, when repressing *chiP*[19]. Significantly, the inactivation of ChiX only occurs under inducing conditions (*chbBC* mRNA in excess), whereas, when the *chb* operon is uninduced and transcribed at a low basal level, ChiX represses *chbC* gene expression (ChiX in excess)[36]. The maintenance of some low-level expression of the *chb* operon may be important to enable some chitosugars to reach the cytoplasm and trigger induction.

Subsequent reports of bacterial mRNAs that compete for regulatory sRNAs suggest that ceRNAs can form the basis of a multi-mRNA network, in which derepression

Box 1 | The ceRNA hypothesis: competition for microRNAs promotes mRNA crosstalk

MicroRNAs (miRNAs) are 21–23 nucleotide RNAs that are involved in gene silencing in animals and plants by guiding effector proteins to the 3′ untranslated region (UTR) of mRNAs, which leads to the repression of translation and/or destabilization of the mRNAs[23]. miRNAs base pair with RNA sequences, known as miRNA response elements (MREs), that can be as short as 6–8 nucleotides and that are found in hundreds of different RNAs from the coding and non-coding portions of the genome. The conventional view that individual miRNAs are able to silence all of the transcripts that contain their cognate MREs has been challenged by the competing endogenous RNA (ceRNA) hypothesis and the work that led to its development. Seitz first argued that the multitude of low-affinity MREs that have been identified by computational approaches were likely to be 'pseudotargets' that are competitive inhibitors of miRNA activity that increase the robustness of the regulation of a few 'true' targets[65]. These concepts were further elaborated by Pandolfi and colleagues into the ceRNA hypothesis, which proposes the existence of a global regulatory network whereby RNA transcripts use MREs to communicate with each other through competition for shared cognate miRNAs[28]. To illustrate the concept that underlies the ceRNA hypothesis, consider a hypothetical example in which a protein-coding RNA and a non-coding RNA share a set of MREs and thus compete for the same set of miRNAs (see the figure). The protein-coding gene for RNA X is transcribed at an invariant rate, whereas transcription of the non-coding gene for RNA Y can be either activated or repressed, depending on transcriptional regulation. When transcription of RNA Y is repressed (or uninduced), miRNAs are present at abundances in excess of their MRE targets; consequently, both RNA X and RNA Y are completely silenced (see the figure, part **a**). However, when RNA Y transcription is derepressed (or induced), the increase in RNA Y levels causes the miRNA pool to be titrated out, so that the MRE targets are now present at abundances in excess of their cognate miRNAs; consequently, post-transcriptional silencing of RNA X is alleviated (see the figure, part **b**). More complex situations that involve several RNAs and partially overlapping MRE repertoires are expected to produce a wide range of combinatorial responses.



can be coordinated by a single sRNA sponge. GcvB, a ~200 nucleotide sRNA that is highly conserved in Enterobacteriaceae, downregulates several mRNAs that encode proteins that are involved in amino acid uptake or biosynthesis[38]. Among the targets of GcvB are the transcription products of the *gltIJKL* operon, which encodes the glutamate/aspartate (Glu/Asp) ABC transporter. The *gltIJKL* operon is transcribed into two mRNAs: a longer transcript that spans the entire operon and a shorter transcript that terminates at a leaky Rho-independent terminator in an intercistronic region between *gltI* and *gltJ*. GcvB represses *gltI* translation in both the longer and shorter transcripts by forming base-pair interactions with a sequence in the 5′ UTR of *gltI*. The shorter transcript is specifically cleaved by RNase E at a site approximately 150 nucleotides from its 3′ end and the released RNA fragment, which is named SroC, is stably maintained in the cell through its association with Hfq[39]. Experiments that investigated whether SroC has a regulatory role revealed that SroC has the ability to sequester GcvB by forming base-pair interactions[39]. SroC behaves as a true sponge by not only sequestering GcvB but also promoting its degradation, thereby decreasing the ability of mRNA targets to compete for GcvB. Furthermore, as GcvB is a master regulator of several mRNAs, the targeting of GcvB by SroC is able to derepress an entire regulon[39] (FIG. 2b).

The mode of action of SroC is non-canonical in that it does not involve target mimicry. Instead, SroC base pairs with two separate regions in the GcvB sequence that are relatively far from each other and do not, in either case, correspond to the region that is used by GcvB to form base-pair interactions with most, although not all[40,41], of its targets. Thus, unlike the ChiX system, in which the ceRNA is an effective regulator only when present above a threshold abundance, SroC might be effective even at a low concentration (especially if it is able to interact with GcvB molecules that are already paired with mRNA targets) and, as such, its activity may be less susceptible than conventional ceRNAs to the expression levels of mRNAs with which it is competing for GcvB. Consequently, crosstalk between SroC and competing mRNAs would be unidirectional, as SroC would regulate these mRNAs but not vice versa, which contrasts with the bidirectional crosstalk that would be expected to occur for ceRNAs that use target mimicry. As SroC is derived from a transcript that is itself repressed by GcvB, the degradation of GcvB that is promoted by SroC establishes a feed-forward loop whereby expression of the *gltI* mRNA upregulates the expression of all other GcvB targets[39]. The physiological role of this regulatory circuitry remains elusive, although the link with glutamate, which is a key intermediate in nitrogen assimilation[42], might suggest that the main role of SroC is to coordinate a response to nitrogen limitation. SroC is only produced from transcripts that are prematurely terminated at the *gltI–gltJ* intercistronic boundary; that is, transcripts that do not contribute to the uptake of glutamate. If termination efficiency were to increase at low levels of glutamate, this would be a mechanism for boosting SroC expression and, in turn, would promote expression of alternative nitrogen sources through GcvB sponging.

An additional RNA species that competes for GcvB is the AgvB sRNA, which is encoded by two prophages in

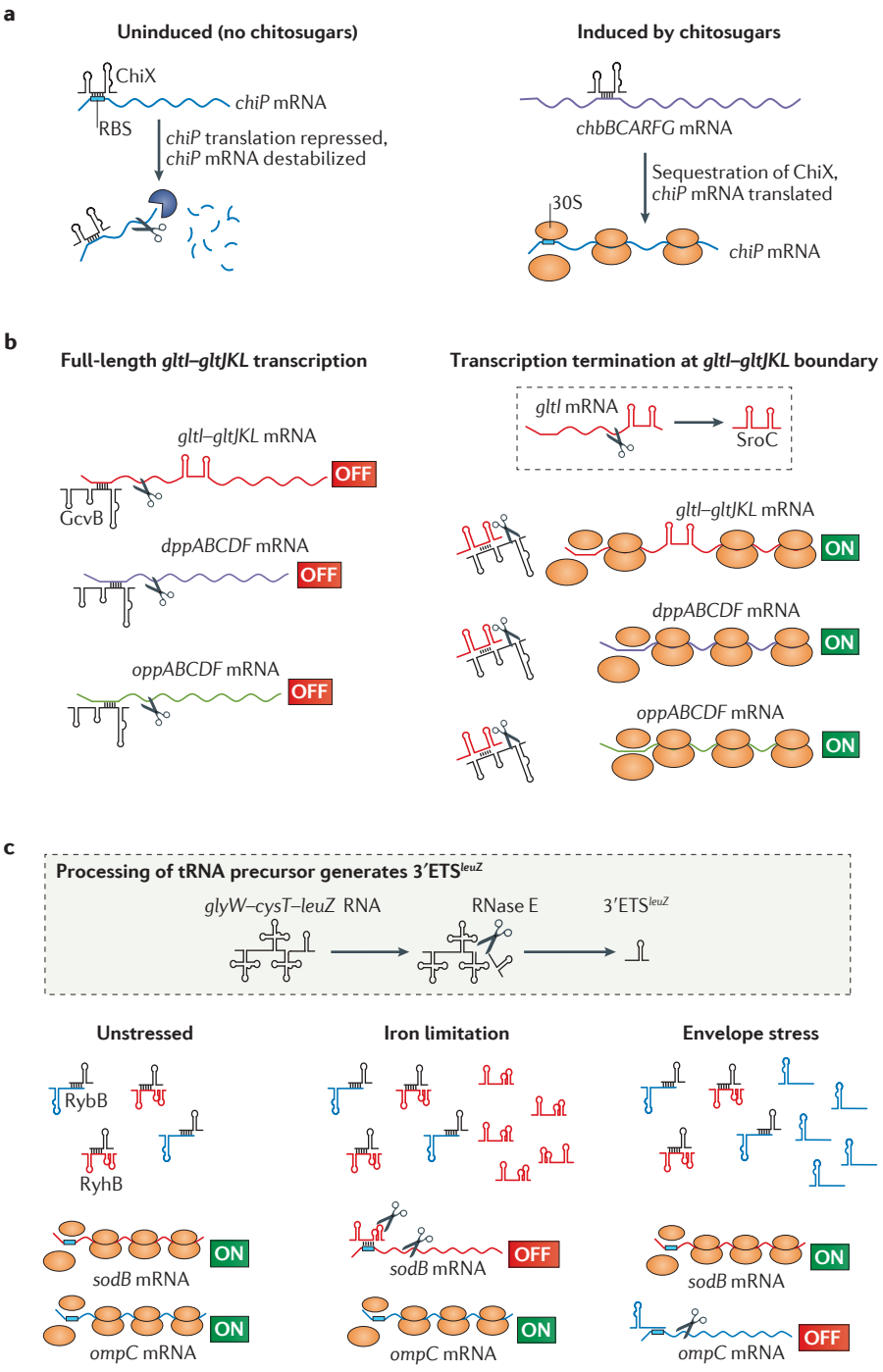


Figure 2 | **RNAs competing for a shared sRNA regulator. a** | Regulation of chitosugar uptake in *Salmonella enterica* and *Escherichia coli*. The gene that encodes the main chitoporin (*chiP*) is transcribed at a relatively high basal level under all conditions; however, if no chitosugars are present, the synthesis of ChiP is prevented by the constitutively produced ChiX small RNA (sRNA), which forms base-pair interactions with a sequence in the ribosomal binding site (RBS) of *chiP* mRNA, thereby inhibiting translation. Transcription of the *chbBCARFG* operon, which encodes the chitobiose transporter and enzymes that are involved in chitobiose catabolism, is repressed by the *N*-acetylglucosamine repressor (NagC; not shown). In the presence of chitosugars, increasing levels of *N*-acetylglucosamine 6-phosphate (GlcNAc6P) lead to the relief of NagC repression and transcription of the *chbBCARFG* operon. Importantly, the *chbB–chbC* intercistronic region in the *chbBCARFG* operon contains a sequence that can compete with the *chiP* mRNA to pair with ChiX. Therefore, as the *chbBCARFG* mRNA accumulates, ChiX is diverted away from *chiP* mRNA by the alternative pairing interaction and is degraded. Note that ChiX binds to its targets while associated with Hfq (not shown). **b** | Target-mediated derepression of the GcvB regulon. The ~200 nucleotide GcvB sRNA pairs with, and inhibits translation of, several mRNAs that encode amino acid and peptide transporters, including those that are produced by transcription of the *gltIJKL*, *dppABCDF* and *oppABCDF* operons. The untranslated mRNAs that are targeted by GcvB are destabilized as a result of degradation by RNase E. A shorter *gltIJKL*-derived RNA is generated by transcription termination at a site within the *gltI–gltJ* intercistronic spacer sequence. RNase E cleavage of this shorter transcript produces the ~150 nucleotide SroC RNA (inset), which competes with GcvB targets to capture GcvB by base pairing and promotes its degradation, thereby activating the translation of the entire GcvB regulon. Note that GcvB binds to its targets while associated with Hfq (not shown). **c** | Sponge activity of an RNA that is derived from a tRNA precursor. RyhB and RybB are regulatory sRNAs that accumulate in response to iron limitation and envelope stress, respectively, and that help bacteria to survive and recover from these conditions. Owing to transcriptional noise, both sRNAs are produced at significant basal levels even when they are not required. In unstressed cells, an RNA (3′ external transcribed spacer of *leuZ* (3′ETS^{leuZ})) that is produced by RNase E cleavage of the 3′end portion of the *glyW–cysT–leuZ* tRNA precursor (inset) sequesters these unwanted sRNAs through base-pair interactions that outcompete the interactions formed with target mRNAs. However, in response to a specific inducing condition, the production of RyhB or RybB is increased such that the ability of the 3′ETS^{leuZ} RNA to prevent accumulation is saturated. In setting a threshold level of expression that these sRNAs must attain to regulate mRNAs, the 3′ETS^{leuZ} RNA increases the robustness of the regulatory response. Superoxide dismutase (*sodB*) and outer-membrane protein C (*ompC*) mRNAs are shown as representative examples of target mRNAs.

the genome of an enterohaemorrhagic strain of *E. coli*[43]. Unlike SroC, AgvB can form base-pair interactions with the main GcvB seed sequence and thus competes directly with endogenous mRNA targets for GcvB. Through such competition, the overexpression of AgvB was able to derepress *dppA*, which encodes the periplasmic binding component of the dipeptide ABC transporter and is a major target of GcvB in *E. coli* and *S. enterica*. Intriguingly, deleting its two copies of *agvB* significantly reduced the ability of the *E. coli* strain to proliferate in mucus from the bovine intestinal tract, which suggests that AgvB improves the fitness of the strain in this environment[43], although the molecular basis that underlies the association between *agvB* and fitness is unknown. One possible explanation might be that intestinal mucus is rich in nutrients that are taken up by GcvB-repressed transporters, in which case derepression of these transporters would confer a selective advantage.

Crosstalk between sRNAs. The small RNAs RybB and RyhB are central regulators of two major homeostatic pathways in both *E. coli* and *S. enterica*, with RybB regulating a cell envelope stress response and RyhB regulating iron homeostasis. Transcription of *rybB* requires the activation of the alternative sigma factor σ^E by folding defects in outer membrane proteins (OMPs); RybB inhibits translation of the mRNAs that encode some of the major OMPs and in doing so suppresses the σ^E-activating signal and thus its own transcription[44,45]. By contrast, transcription of *ryhB* is repressed by the ferric-uptake regulator (Fur) under normal conditions, but the sRNA becomes highly transcribed when intracellular iron levels are depleted. RyhB silences several mRNAs that encode non-essential iron-binding proteins[46,47] and the sRNA also activates iron uptake by stimulating translation of mRNAs that are involved in siderophore biosynthesis or transport[48,49]. Thus, RyhB uses a dual approach to replenish the intracellular iron pool, which, in turn, restores Fur-mediated repression of the *ryhB* gene.

Recently, sequences from RybB or RyhB were used as baits to identify new targets that co-purify with these sRNAs. In addition to confirming many previously reported targets, the search revealed an unlikely new target that was co-purified with the sequences from both sRNAs: the 3′ external transcribed spacer of *leuZ* (3′ETS^{leuZ})[50]. 3′ETS^{leuZ}, which was also shown to bind to Hfq, is derived from the *glyW–cysT–leuZ* operon, which comprises three tRNA genes that are co-transcribed as a tricistronic precursor that is subsequently cleaved into the mature forms of the three tRNAs; 3′ETS^{leuZ} represents ~50 nucleotides of the 3′ terminal segment of the tRNA precursor and is cleaved from the longer transcript by RNase E during processing. In the absence of inducing signals, 3′ETS^{leuZ} sequesters the RybB and/or RyhB molecules that are transcribed as a result of stochastic fluctuations of promoter activity (FIG. 2c). However, 3′ETS^{leuZ} does not interfere with the accumulation of either of the sRNAs under conditions that activate their promoters. In other words, 3′ETS^{leuZ} antagonizes RybB and/or RyhB only when present in large stoichiometric excess and thus sets a concentration threshold that either sRNA must reach to become effective in regulation. Interestingly, the threshold level that is required for either RybB or RyhB to saturate the sponging capacity of 3′ETS^{leuZ} is predicted to vary depending on the level of expression of the other sRNA, as both sRNAs are competing for the same sponge. In targeting both RybB and RyhB, 3′ETS^{leuZ} might physiologically link iron homeostasis to the σ^E-dependent envelope stress response, owing to the regulatory crosstalk that is expected to result from the competition for 3′ETS^{leuZ} by the two sRNAs[50]. This crosstalk between sRNAs, mediated by a sponge, is the reverse of the more commonly reported ceRNA regulatory mechanism, in which crosstalk between mRNA sponges is mediated by an sRNA. The study found a high degree of conservation of spacer sequences in other tRNA precursors, which suggests that buffering against stochastic variations in sRNA levels that result from transcriptional noise may be a widespread characteristic of these molecules[50]. However, whether sponging by spacer sequences is also commonly used to mediate crosstalk between sRNA targets is not known, and cannot be investigated until any such targets have been identified.

Competition for RNA-binding proteins

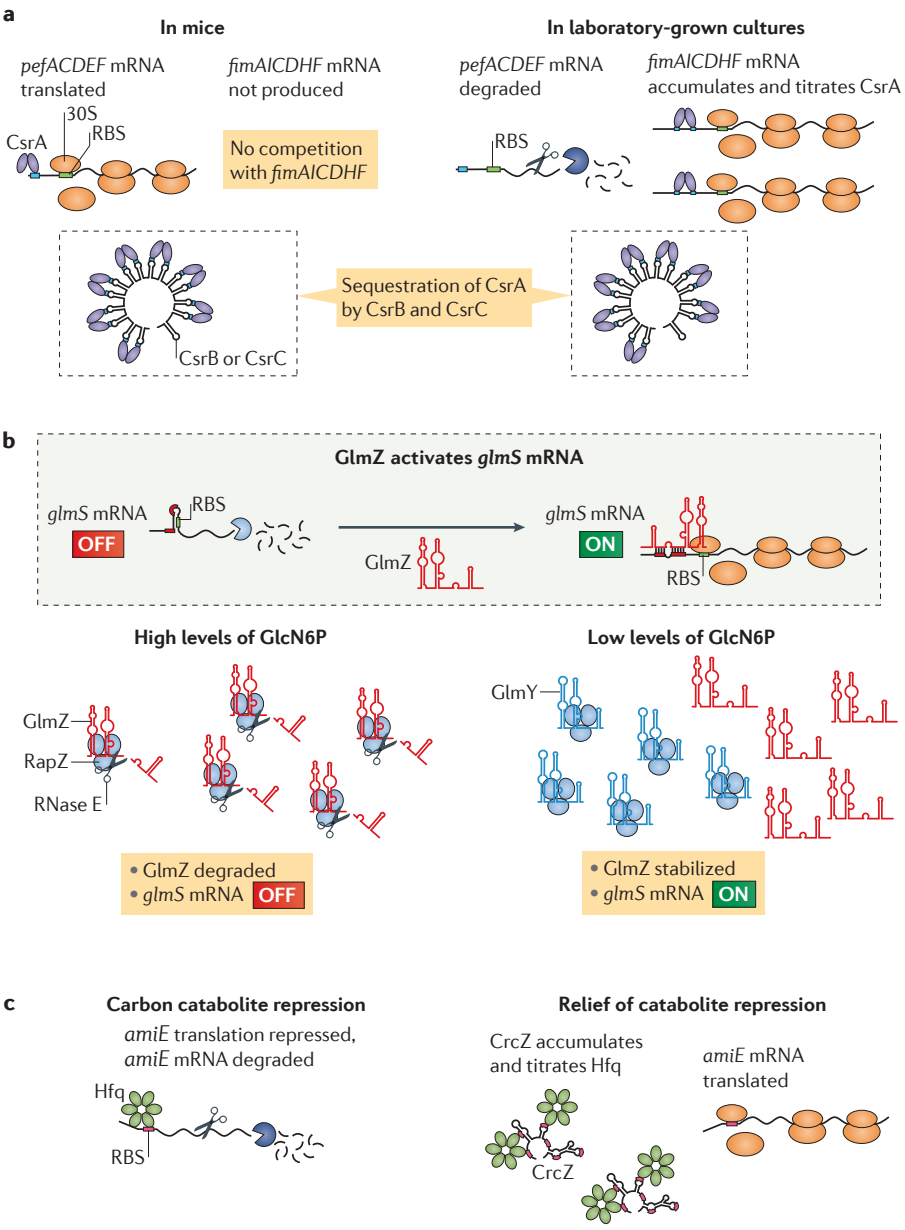
Although studies of ceRNAs in eukaryotic cells have focused on the potential for crosstalk between RNAs that compete for the same RNA sponge, an increasing amount of evidence indicates that an alternative form of crosstalk between ceRNAs in bacteria is mediated by competition for an

RNA-binding protein. As with competition for RNA sponges, ceRNAs that compete for RNA-binding proteins can include both mRNAs and sRNAs.

Crosstalk between mRNAs. A representative example of crosstalk that is mediated by competition for an RNA-binding protein involves competition for the global post-transcriptional regulator CsrA (carbon storage regulator) during fimbrial gene expression in *S. enterica*. The main regulation of cellular CsrA availability occurs through its sequestration by the sRNAs CsrB and CsrC[51]. However, in the regulation of fimbrial gene expression, the pool of CsrA that is not sequestered by CsrB and CsrC was recently shown to be titrated by a ceRNA, which revealed a fundamental role for CsrA as a mediator of crosstalk between the transcripts of the fimbrial operons *fimAICDHF* and *pefACDEF*[52]. CsrA can bind to sites in the 5′ UTR of both the *fimAICDHF* and *pefACDEF* transcripts but, of the two, only *pefACDEF* is directly affected by CsrA binding. More specifically, CsrA binding activates the *pefACDEF* transcript (by an unknown mechanism), but has no effect on the *fimAICDHF* transcript[52]. Instead, the role of the CsrA binding site in the 5′ UTR of *fimAICDHF* is to function as a sponge that competes with *pefACDEF* for CsrA binding. Thus, titration of CsrA by the *fimAICDHF* 5′ UTR cooperates with sequestration of CsrA by the sRNAs CsrB and CsrC to repress the *pefACDEF* operon (FIG. 3a).

Two key features make the CsrA-mediated regulatory crosstalk between *fimAICDHF* and *pefACDEF* possible: first, the exceptionally high rate of transcription of *fimAICDHF* establishes a marked stoichiometric imbalance in which the CsrA-titrating sequence in the *fimAICDHF* transcript accumulates to a level between ~100-fold and 1,000-fold higher than that of most CsrA-regulated transcripts; and, second, the low binding affinity of CsrA for its binding site in the *pefACDEF* transcript. It is significant that stoichiometry and binding affinity are also fundamental to sRNA-mediated regulatory crosstalk, as these are both mechanisms that enable a ceRNA to outcompete other RNAs, whether for a shared regulatory sRNA or a shared RNA-binding protein.

It should be noted that CsrA regulates many pathways other than fimbrial gene expression, notably pathways that are associated with growth-phase transitions or environmental adaptation. Therefore, an intriguing prediction is that the expression of the *fimAICDHF* operon might cross-regulate other mRNAs that are under the control of CsrA, in addition to the *pefACDEF* transcript. One such transcript is expressed from the *flhDC* locus and is stabilized by CsrA. As predicted, deletion of the *fimAICDHF* operon resulted in an increase in the levels of *flhD* mRNA; however, the phenotype was only partially complemented by a plasmid that carried the 5′ UTR of the *fimAICDHF* transcript[52]. Overall, one might envision that the expression of *fimAICDHF* will only cross-regulate those mRNAs that, similarly to *pefACDEF*, have a low binding affinity for CsrA. These might represent a relatively small proportion of CsrA-regulated transcripts in the cell, which would enable regulatory crosstalk to occur specifically between fimbrial gene clusters without perturbing CsrA regulation of transcripts with other functions.



Crosstalk between sRNAs. Glucosamine-6-phosphate (GlcN6P) is a precursor in the synthesis of peptidoglycan and of the lipid A moiety of lipopolysaccharides in Gram-negative bacteria[53,54]. In the absence of exogenous amino sugars, GlcN6P is synthesized *de novo* from fructose-6-phosphate and glutamine in a reaction that is catalysed by glucosamine-6-phosphate synthase (GlmS). In *E. coli*, *glmS* is activated both

Figure 3 | **RNAs competing for binding to a protein. a** | Competition as the basis for hierarchical control of fimbrial gene expression in *Salmonella enterica*. The plasmid-borne fimbrial operon *pefACDEF* is stably expressed by *S. enterica* in mice but not in laboratory-grown cultures. The regulatory switch operates post-transcriptionally and involves competition for an RNA-binding protein (carbon storage regulator (CsrA)) with the transcript from a second fimbrial locus, *fimAICDHF*. Synthesis of the fimbriae requires CsrA to bind to a site near the 5′end of the *pefA* 5′ untranslated region (UTR) to activate (by an unknown mechanism) the *pefACDEF* mRNA; however, CsrA can also bind to the 5′ UTR of *fimA*, in which two strong binding sites confer greater affinity for CsrA than the binding site in the 5′ UTR of *pefA*. Although *fimA* is not expressed in mice, in laboratory-grown *S. enterica* cultures, *fimAICDHF* transcription leads to the presence of many copies of the 5′ UTR of *fimA* and thus sequestration of CsrA. The depletion of the free CsrA pool causes the loss of *pefACDEF* activation, which leads to the degradation of *pefACDEF* mRNA (presumably by RNase E and exonucleases). In both environments, the small RNAs (sRNAs) CsrB and CsrC also compete with the target site in the 5′ UTR of *pefA* to sequester CsrA. **b** | Competition for an RNase E adaptor protein. In *Escherichia coli*, glucosamine-6-phosphate (GlcN6P) synthase (*glmS*) mRNA is naturally translated at a low efficiency, owing to a secondary structure that sequesters the ribosome binding site (RBS), and consequently degraded by RNase E and exonucleases; however, the sRNA GlmZ can form base-pair interactions to denature this secondary structure, thereby activating translation. In the presence of high levels of GlcN6P, GlmZ-mediated activation of *glmS* mRNA is prevented by RapZ, which is an adaptor protein that guides RNase E to cleave GlmZ such that the *glmS*-binding site is removed. A decrease in the concentration of GlcN6P leads to the accumulation of GlmY, which is an sRNA that can efficiently bind to RapZ. By successfully competing with GlmZ for RapZ binding, GlmY prevents the cleavage of GlmZ and thus enables GlmZ to activate translation of *glmS*. **c** | Regulation of carbon catabolite repression through Hfq sequestration in *Pseudomonas aeruginosa*. Key steps in the control of carbon catabolite repression in *P. aeruginosa* occur post-transcriptionally and involve Hfq binding to the RBS of catabolite-responsive mRNAs (such as acylamide amidohydrolase (*amiE*) mRNA), thereby inhibiting translation and leading to mRNA destabilization (presumably owing to degradation by RNase E and exonucleases). Relief of carbon catabolite repression occurs when a less-favoured carbon source is used and results from the accumulation of CrcZ, which is a regulatory RNA that has several Hfq binding sites (red dots) that compete with Hfq binding sites in catabolite-responsive mRNAs. Thus, CrcZ is a sponge that sequesters Hfq to relieve carbon catabolite repression.

transcriptionally and post-transcriptionally in response to a decrease in the level of GlcN6P in the cell[55], with post-transcriptional activation mediated by GlmZ, which is a 207 nucleotide sRNA that forms base-pair interactions with a sequence in the 5′ UTR of *glmS* mRNA. GlmZ promotes *glmS* translation by antagonizing the formation of a secondary structure in the 5′ UTR that inhibits translation initiation (FIG. 3b). Under non-inducing conditions, when GlcN6P levels are high, GlmZ is inactivated by a specific endonucleolytic cleavage event that excises the *glmS*-binding region and that depends on the presence of an adaptor protein, RapZ, which is an RNA-binding protein that guides RNase E to the cleavage site[56,57].

The activation of *glmS* expression is made possible by a mechanism that blocks the cleavage of GlmZ by RNase E–RapZ during GlcN6P depletion. A key component of this switching mechanism is a second sRNA, GlmY, which is structurally related to GlmZ[58]. GlmY, which accumulates as GlcN6P levels decrease, uses its structural similarity to compete with GlmZ for binding to RapZ; however, unlike GlmZ, GlmY is not cleaved by RNase E. By sequestering RapZ, GlmY enables GlmZ to escape degradation by RNase E, and the stabilized GlmZ can then activate *glmS* to upregulate the synthesis of GlcN6P and thus maintain GlcN6P homeostasis. In summary, although GlmZ is directly responsible for the activation of *glmS*, the regulatory switch that leads to this activation involves crosstalk between GlmY and GlmZ that is mediated by competition for the RapZ RNA-binding protein[58].

Crosstalk mediated by Hfq. Catabolite repression denotes the inhibition of the synthesis of enzymes that are involved in the use of less-preferred carbon sources when a preferred source is available. In *P. aeruginosa*, key steps in catabolite repression occur post-transcriptionally and in response to changes in the levels of CrcZ, which is a 407 nucleotide non-coding RNA[59] that reaches maximal levels when bacteria grow in the presence of poor carbon sources and decreases following a shift to a preferred source. CrcZ contains several copies of the so-called 'catabolite activity motif' (AANAANAA), which is also found in sites proximal to the translation initiation codons of genes that are repressed during catabolite repression[59].

For some time, the catabolite activity motif was thought to be the target of catabolite repression control protein (Crc), which is the master regulator of catabolite repression in *P. aeruginosa*. As such, it was thought that CrcZ would regulate mRNAs that contain the catabolite activity motif by titrating Crc, similarly to the titration of CsrA by the sRNAs CsrB and CsrC and their homologues in *E. coli* and *S. enterica* (see above)[51,60]. According to this model, Crc would repress translation of target mRNAs by binding to catabolite activity motifs near translation start sites and CrcZ would relieve this repression by competing with these mRNAs for Crc. However, recent work has made a crucial revision to this model: the protein that binds to catabolite activity motifs is not Crc but Hfq, which is able to directly repress (that is, without the participation of sRNAs) mRNAs that contain catabolite activity motifs near their translation start sites[12]. Under inducing conditions, upregulated CrcZ is a decoy that sequesters Hfq to relieve the repression of mRNAs that are now outcompeted for Hfq binding to their catabolite activity motifs (FIG. 3c). Thus, competition for Hfq mediates regulatory crosstalk between CrcZ and mRNAs that are repressed during catabolite repression. The role of Crc in the revised model remains unclear, although it seems that Crc is nevertheless involved in the regulation of catabolite repression and that this may occur through the formation of a ternary complex with Hfq and CrcZ[61].

As in the case of fimbrial regulation by CsrA (FIG. 3a), the question arises as to whether Hfq sequestration by CrcZ (or its homologues CrcX and CrcY, which have been described in other *Pseudomonas* species) has pleiotropic effects on processes that are unrelated to carbon metabolism, in particular processes that involve Hfq-dependent sRNAs, as might be predicted. Unfortunately, our knowledge of these sRNAs in *Pseudomonas* spp. is currently too limited to enable this question to be answered[14].

The systems biology of bacterial ceRNAs

Microorganisms in natural habitats must constantly adjust their metabolism and developmental programmes as a function of countless combinations of environmental parameters. Furthermore, the regulatory response to a given condition needs to be tuned to, and physiologically compatible with, the responses to other conditions. We suggest that the interplay of transcriptional regulation and crosstalk between ceRNAs could provide a mechanism for communication between the regulatory pathways that control these responses. Indeed, several mRNAs,

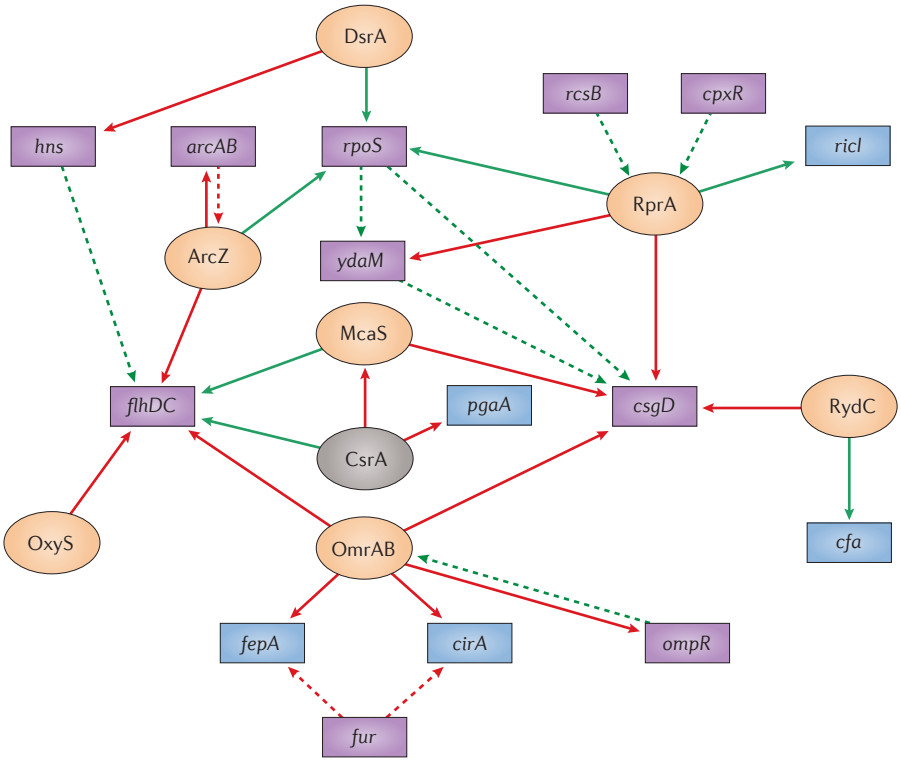


Figure 4 | **Interconnection network in which ceRNAs may link disparate pathways in *Escherichia coli* and *Salmonella enterica*.** Regulation by small RNAs (sRNAs) that may involve competing endogenous RNAs (ceRNAs) has been reported for several disparate pathways in *Escherichia coli* and *Salmonella enterica*, including the pathways that regulate stationary phase growth, biofilm formation or motility. Competition involving RNAs that are common to several pathways may lead to the establishment of a wider regulatory network, with nodes that include mRNAs that encode transcription factors or other regulatory proteins (purple boxes), mRNAs that encode proteins with functions that are related to the output of the pathway (blue boxes), regulatory sRNAs (orange ovals) and regulatory RNA-binding proteins, such as CsrA (grey oval). Connections in the network involve the activation of transcription (green dashed arrows), the repression of transcription (red dashed arrows), post-transcriptional activation (green continuous arrows) or post-transcriptional repression (red continuous arrows). Some components of the network have been omitted for simplicity. The network shown in the figure was constructed using the data from REFS 3,63,64,71–75. *cfa*, cyclopropane-fatty-acyl-phospholipid synthase; *cirA*, colicin IA outer membrane receptor; *csrA*, carbon storage regulator; *fepA*, ferrienterobactin outer membrane transporter; *fur*, ferric-uptake regulator.

including those that encode transcription factors or regulators, such as RpoS, PhoP, CsgD, OmpR, FlhDC, Lrp and LuxO, have been shown to be targeted by more than one sRNA, and each of these sRNAs, in turn, has several other targets[3,62,63]. Such mRNAs and sRNAs could be ceRNAs that form nodes that link together networks of the transcriptome that are otherwise separated, thus enabling the transmission of regulatory responses. For example, an increase in the levels of an mRNA in a given network might alleviate the repression of mRNAs from another network that is linked to the first by an sRNA node, as a result of mRNA-mediated sRNA sponging. One system in which this example could apply is the mechanism that links the regulation of stationary phase growth, biofilm formation and envelope stress in *E. coli* and *S. enterica* (FIG. 4). In this regulatory network, the central regulator is the *csgD* mRNA, which encodes the transcriptional activator that is required for the production of curli fimbriae and the biosynthesis of cellulose. Furthermore, *csgD* mRNA is targeted by several sRNAs, including RprA, McaS, OmrA and OmrB, and as such is a hub for signal integration[3,62]. It was proposed that during entry to stationary phase or during the initial stages of biofilm formation, which are conditions in which *csgD* mRNA accumulates to high levels, the *csgD* mRNA is a sponge for RprA that outcompetes all other RprA targets. In *S. enterica*, RprA positively regulates approximately 30 genes, including the gene that encodes the alternative sigma factor RpoS, and negatively regulates a similar number of genes[64]. Thus, the accumulation of *csgD* mRNA may result in the activation and repression of mRNAs in a large regulon. One may also speculate that *csgD* is a sponge for the McaS and/or OmrA and OmrB sRNAs, which have partially overlapping roles in controlling the switch between planktonic and sessile lifestyles and, in the case of OmrA and OmrB, regulate several mRNAs that encode outer membrane proteins, including iron transporters. Therefore, if *csgD* does indeed function as a sponge for these sRNAs, the effects of the accumulation of *csgD* mRNA would be expected to propagate to flagellar regulation and iron uptake[64] (FIG. 4).

How pervasive is the contribution of ceRNAs to bacterial physiology? Despite the increasing number of examples of bacterial ceRNAs that have been reported, we believe that the phenomenon remains underestimated. The classic view of post-transcriptional regulation by bacterial sRNAs focuses on the ability of some of the best-characterized sRNAs to control large regulons of mRNAs. However, this view is largely based on evidence obtained from overexpressing sRNA genes from plasmid vectors; that is, conditions that drastically alter the sRNA/mRNA stoichiometry. Even under these artificially high sRNA/mRNA ratios, the sRNA-induced changes in the levels of many mRNAs are often not higher than twofold or threefold, which casts doubt on the description of these mRNAs as bona fide sRNA targets. Instead, the modest changes in abundance raise the possibility that these mRNAs are in fact pseudotargets that function as competitive inhibitors of sRNA activity, similarly to what has been proposed for some mRNA targets of eukaryotic miRNAs[63] (BOX 1); these pseudotargets may have a role in increasing the robustness of the regulatory response and/or linking the response to other networks[21,65].

The nodes that are formed in ceRNA networks would also include the RNA-binding proteins CsrA and Hfq as global regulators that have been shown to mediate crosstalk between ceRNAs. Both proteins bind to a wide range of RNA ligands with a variable degree of affinity, and this variation enables RNA ligands that have higher binding affinities, under certain conditions, to sequester a sufficient amount of the protein to limit its availability for RNA ligands that have weaker binding affinities. For CsrA, this use of variable binding affinities to achieve a sponging effect is exemplified by the mechanism of fimbrial regulation (see above), as well as

by the regulation of the *pgaABCD* locus that is involved in biofilm formation in *E. coli*. CsrA binds to several sites in the 5′ UTR of *pgaA*, thereby inhibiting translation and promoting premature transcription termination through distinct mechanisms[66,67]. The sRNA McaS, which normally targets mRNAs, can also bind to CsrA and is able to outcompete the *pgaA* leader mRNA for CsrA binding. Thus, CsrA mediates crosstalk between McaS and the *pgaA* leader sequence to relieve repression of the *pgaABCD* locus. For Hfq, the example of CrcZ[12] (see above) may not be unique; in *E. coli* and *S. enterica*, the availability of Hfq is a limiting factor for sRNA function[68–70]. One may predict that variations in the levels of one or more sRNAs, or mRNAs, that bind to Hfq with high affinities will affect the abilities of other sRNAs to bind to Hfq and thus regulate their targets.

As our knowledge of the roles of regulatory RNAs in transcriptional networks improves, it will become increasingly possible to identify those members of the network that function as ceRNAs. Experimentally altering the *in vivo* concentrations and/or synthesis rates of putative ceRNAs will enable an evaluation of how changes in the abundance of each ceRNA affect RNAs in the network, as well as RNAs in linked networks. The regulatory potential of ceRNAs could even be used in synthetic biology, with the introduction of chimeric or artificial ceRNAs on the bacterial chromosome that are used to modify an existing network or to connect two previously unconnected networks. Exploring the physiological consequences of such changes and designing experiments that are based on predictive mathematical modelling will help to expand our knowledge of the mechanisms and systems biology of ceRNAs, and to understand how pervasive this recently discovered biological strategy is, given its conservation in most living organisms, in the regulation of bacterial transcriptomes.

Concluding remarks

In this Opinion article, we have reviewed evidence that indicates that RNAs that compete for shared sRNA or protein regulators form an important component of gene regulation in several key biological processes. Through competition, RNAs can cross-regulate the translation and/or stability of other RNAs. The main parameters that determine the strength of regulation by ceRNAs are the stoichiometry of the competitors, their relative affinities for the regulator, and, in the case of sRNA-mediated competition, the fate of the sRNA after base pairing with cognate RNAs. All of these parameters can vary continuously over a wide range, which makes the regulatory architecture formed by ceRNAs particularly suited to the establishment of hierarchical crosstalk between genes and networks. We have argued that this crosstalk may be of primary importance in enabling bacterial cells to integrate a combinatorial array of environmental signals and to process these signals into a unitary and coherent adaptive response. We expect that additional examples that corroborate the role of ceRNAs in bacterial physiology and environmental adaptation will be obtained in the near future and that bacterial ceRNAs will soon constitute an exciting new area of investigation for experimentalists and theoreticians alike.

Lionello Bossi and Nara Figueroa-Bossi are at the Institute for Integrative Biology of the Cell (I2BC), Centre National de la Recherche Scientifique (CNRS), The French Alternative Energies and Atomic Energy Commission (CEA), University Paris-Saclay, 1 Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France.

Correspondence to L.B.
lionello.bossi@i2bc.paris-saclay.fr

doi:10.1038/nrmicro.2016.129
Published online 19 Sep 2016

1. Felden, B., Vandenesch, F., Bouloc, P. & Romby, P. The *Staphylococcus aureus* RNome and its commitment to virulence. *PLoS Pathog.* **7**, e1002006 (2011).
2. Gottesman, S. & Storz, G. Bacterial small RNA regulators: versatile roles and rapidly evolving variations. *Cold Spring Harb. Perspect. Biol.* **3**, a003798 (2011).
3. Mika, F. & Hengge, R. Small regulatory RNAs in the control of motility and biofilm formation in *E. coli* and *Salmonella*. *Int. J. Mol. Sci.* **14**, 4560–4579 (2013).
4. Papenfort, K. & Vogel, J. Small RNA functions in carbon metabolism and virulence of enteric pathogens. *Front. Cell. Infect. Microbiol.* **4**, 91 (2014).
5. Storz, G., Vogel, J. & Wassarman, K. M. Regulation by small RNAs in bacteria: expanding frontiers. *Mol. Cell* **43**, 880–891 (2011).
6. Massé, E., Escorcia, F. E. & Gottesman, S. Coupled degradation of a small regulatory RNA and its mRNA targets in *Escherichia coli*. *Genes Dev.* **17**, 2374–2383 (2003).
7. Dreyfus, M. Killer and protective ribosomes. *Prog. Mol. Biol. Transl. Sci.* **85**, 423–466 (2009).
8. Bossi, L., Schwartz, A., Guillemardet, B., Boudvillain, M. & Figueroa-Bossi, N. A role for Rho-dependent polarity in gene regulation by a noncoding small RNA. *Genes Dev.* **26**, 1864–1873 (2012).
9. Hopkins, J. F., Panja, S. & Woodson, S. A. Rapid binding and release of Hfq from ternary complexes during RNA annealing. *Nucleic Acids Res.* **39**, 5193–5202 (2011).
10. Panja, S. & Woodson, S. A. Hfq proximity and orientation controls RNA annealing. *Nucleic Acids Res.* **40**, 8690–8697 (2012).
11. Vogel, J. & Luisi, B. F. Hfq and its constellation of RNA. *Nat. Rev. Microbiol.* **9**, 578–589 (2011).
12. Sonnleitner, E. & Bläsi, U. Regulation of Hfq by the RNA CrcZ in *Pseudomonas aeruginosa* carbon catabolite repression. *PLoS Genet.* **10**, e1004440 (2014).
13. Desnoyers, G. & Massé, E. Noncanonical repression of translation initiation through small RNA recruitment of the RNA chaperone Hfq. *Genes Dev.* **26**, 726–739 (2012).
14. Salvail, H., Caron, M. P., Bélanger, J. & Massé, E. Antagonistic functions between the RNA chaperone Hfq and an sRNA regulate sensitivity to the antibiotic colicin. *EMBO J.* **32**, 2764–2778 (2013).
15. Bohn, C., Rigoulay, C. & Bouloc, P. No detectable effect of RNA-binding protein Hfq absence in *Staphylococcus aureus*. *BMC Microbiol.* **7**, 10 (2007).
16. Liu, Y. *et al.* Hfq is a global regulator that controls the pathogenicity of *Staphylococcus aureus*. *PLoS ONE* **5**, e13069 (2010).
17. Rochat, T., Bouloc, P., Yang, Q., Bossi, L. & Figueroa-Bossi, N. Lack of interchangeability of Hfq-like proteins. *Biochimie* **94**, 1554–1559 (2012).
18. Papenfort, K. & Vanderpool, C. K. Target activation by regulatory RNAs in bacteria. *FEMS Microbiol. Rev.* **39**, 362–378 (2015).
19. Overgaard, M., Johansen, J., Moller-Jensen, J. & Valentin-Hansen, P. Switching off small RNA regulation with trap-mRNA. *Mol. Microbiol.* **73**, 790–800 (2009).
20. Figueroa-Bossi, N., Valentini, M., Malleret, L., Fiorini, F. & Bossi, L. Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target. *Genes Dev.* **23**, 2004–2015 (2009).
21. Jost, D., Nowojewski, A. & Levine, E. Small RNA biology is systems biology. *BMB Rep.* **44**, 11–21 (2011).
22. Levine, E., Zhang, Z., Kuhlman, T. & Hwa, T. Quantitative characteristics of gene regulation by small RNA. *PLoS Biol.* **5**, e229 (2007).
23. Bartel, D. P. MicroRNAs: target recognition and regulatory functions. *Cell* **136**, 215–233 (2009).
24. Ebert, M. S., Neilson, J. R. & Sharp, P. A. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat. Methods* **4**, 721–726 (2007).
25. Franco-Zorrilla, J. M. *et al.* Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* **39**, 1033–1037 (2007).
26. Ebert, M. S. & Sharp, P. A. Emerging roles for natural microRNA sponges. *Curr. Biol.* **20**, R858–R861 (2010).
27. Tan, J. Y. & Marques, A. C. The miRNA-mediated cross-talk between transcripts provides a novel layer of posttranscriptional regulation. *Adv. Genet.* **85**, 149–199 (2014).
28. Salmena, L., Poliseno, L., Tay, Y., Kats, L. & Pandolfi, P. P. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* **146**, 353–358 (2011).
29. Denzler, R., Agarwal, V., Stefano, J., Bartel, D. P. & Stoffel, M. Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol. Cell* **54**, 766–776 (2014).
30. Jens, M. & Rajewsky, N. Competition between target sites of regulators shapes post-transcriptional gene regulation. *Nat. Rev. Genet.* **16**, 113–126 (2015).
31. de Giorgio, A., Krell, J., Harding, V., Stebbing, J. & Castellano, L. Emerging roles of competing endogenous RNAs in cancer: insights from the regulation of PTEN. *Mol. Cell. Biol.* **33**, 3976–3982 (2013).
32. Figliuzzi, M., De Martino, A. & Marinari, E. RNA-based regulation: dynamics and response to perturbations of competing RNAs. *Biophys. J.* **107**, 1011–1022 (2014).
33. Bosia, C., Pagnani, A. & Zecchina, R. Modelling competing endogenous RNA networks. *PLoS ONE* **8**, e66609 (2013).
34. Ala, U. *et al.* Integrated transcriptional and competitive endogenous RNA networks are cross-regulated in permissive molecular environments. *Proc. Natl Acad. Sci. USA* **110**, 7154–7159 (2013).
35. Tharanathan, R. N. & Kittur, F. S. Chitin — the undisputed biomolecule of great potential. *Crit. Rev. Food Sci. Nutr.* **43**, 61–87 (2003).
36. Plumbridge, J., Bossi, L., Oberto, J., Wade, J. T. & Figueroa-Bossi, N. Interplay of transcriptional and small RNA-dependent control mechanisms regulates chitosugar uptake in *Escherichia coli* and *Salmonella*. *Mol. Microbiol.* **92**, 648–658 (2014).
37. Plumbridge, J. & Pellegrini, O. Expression of the chitobiose operon of *Escherichia coli* is regulated by three transcription factors: NagC, ChbR and CAP. *Mol. Microbiol.* **52**, 437–449 (2004).

38. Sharma, C. M. *et al.* Pervasive post-transcriptional control of genes involved in amino acid metabolism by the Hfq-dependent GcvB small RNA. *Mol. Microbiol.* **81**, 1144–1165 (2011).
39. Miyakoshi, M., Chao, Y. & Vogel, J. Cross talk between ABC transporter mRNAs via a target mRNA-derived sponge of the GcvB small RNA. *EMBO J.* **34**, 1478–1492 (2015).
40. Coornaert, A., Chiaruttini, C., Springer, M. & Guillier, M. Post-transcriptional control of the *Escherichia coli* PhoQ–PhoP two-component system by multiple sRNAs involves a novel pairing region of GcvB. *PLoS Genet.* **9**, e1003156 (2013).
41. Lee, H. J. & Gottesman, S. sRNA roles in regulating transcriptional regulators: Lrp and SoxS regulation by sRNAs. *Nucleic Acids Res.* **44**, 6907–6923 (2016).
42. Ikeda, T. P., Shauger, A. E. & Kustu, S. *Salmonella typhimurium* apparently perceives external nitrogen limitation as internal glutamine limitation. *J. Mol. Biol.* **259**, 589–607 (1996).
43. Tree, J. J., Granneman, S., McAteer, S. P., Tollervey, D. & Gally, D. L. Identification of bacteriophage-encoded anti-sRNAs in pathogenic *Escherichia coli*. *Mol. Cell* **55**, 199–213 (2014).
44. Johansen, J., Rasmussen, A. A., Overgaard, M. & Valentin-Hansen, P. Conserved small non-coding RNAs that belong to the σ^E regulon: role in down-regulation of outer membrane proteins. *J. Mol. Biol.* **364**, 1–8 (2006).
45. Papenfort, K. *et al.* σ^E-dependent small RNAs of *Salmonella* respond to membrane stress by accelerating global *omp* mRNA decay. *Mol. Microbiol.* **62**, 1674–1688 (2006).
46. Jacques, J. F. *et al.* RyhB small RNA modulates the free intracellular iron pool and is essential for normal growth during iron limitation in *Escherichia coli*. *Mol. Microbiol.* **62**, 1181–1190 (2006).
47. Massé, E., Vanderpool, C. K. & Gottesman, S. Effect of RyhB small RNA on global iron use in *Escherichia coli*. *J. Bacteriol.* **187**, 6962–6971 (2005).
48. Balbontín, R. *et al.* Expression of IroN, the Salmochelin siderophore receptor, requires mRNA activation by RyhB small RNA homologues. *Mol. Microbiol.* **100**, 139–155 (2016).
49. Prevost, K. *et al.* The small RNA RyhB activates the translation of *shiA* mRNA encoding a permease of shikimate, a compound involved in siderophore synthesis. *Mol. Microbiol.* **64**, 1260–1273 (2007).
50. Lalaouna, D. *et al.* A 3′ external transcribed spacer in a tRNA transcript acts as a sponge for small RNAs to prevent transcriptional noise. *Mol. Cell* **58**, 393–405 (2015).
51. Vakulskas, C. A., Potts, A. H., Babitzke, P., Ahmer, B. M. & Romeo, T. Regulation of bacterial virulence by Csr (Rsm) systems. *Microbiol. Mol. Biol. Rev.* **79**, 193–224 (2015).
52. Sterzenbach, T. *et al.* A novel CsrA titration mechanism regulates fimbrial gene expression in *Salmonella typhimurium*. *EMBO J.* **32**, 2872–2883 (2013).
53. Park, J. T. in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (eds Neidhart, F. C. *et al.*) 663–671 (ASM Press, 1987).
54. Raetz, C. R. H. in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (eds Neidhart, F. C. *et al.*) 498–503 (ASM Press, 1987).
55. Kalamorz, F., Reichenbach, B., Marz, W., Rak, B. & Görke, B. Feedback control of glucosamine-6-phosphate synthase GlmS expression depends on the small RNA GlmZ and involves the novel protein YhbJ in *Escherichia coli*. *Mol. Microbiol.* **65**, 1518–1533 (2007).
56. Urban, J. H. & Vogel, J. Two seemingly homologous noncoding RNAs act hierarchically to activate *glmS* mRNA translation. *PLoS Biol.* **6**, e64 (2008).
57. Reichenbach, B., Maes, A., Kalamorz, F., Hajnsdorf, E. & Görke, B. The small RNA GlmY acts upstream of the sRNA GlmZ in the activation of *glmS* expression and is subject to regulation by polyadenylation in *Escherichia coli*. *Nucleic Acids Res.* **36**, 2570–2580 (2008).
58. Göpel, Y., Papenfort, K., Reichenbach, B., Vogel, J. & Görke, B. Targeted decay of a regulatory small RNA by an adaptor protein for RNase E and counteraction by an anti-adaptor RNA. *Genes Dev.* **27**, 552–564 (2013).
59. Sonnleitner, E., Abdou, L. & Haas, D. Small RNA as global regulator of carbon catabolite repression in *Pseudomonas aeruginosa*. *Proc. Natl Acad. Sci. USA* **106**, 21866–24871 (2009).
60. Lapouge, K., Schubert, M., Allain, F. H. & Haas, D. Gac/Rsm signal transduction pathway of γ-proteobacteria: from RNA recognition to regulation of social behaviour. *Mol. Microbiol.* **67**, 241–253 (2008).
61. Moreno, R. *et al.* The Crc and Hfq proteins of *Pseudomonas putida* cooperate in catabolite repression and formation of ribonucleic acid complexes with specific target motifs. *Environ. Microbiol.* **17**, 105–118 (2015).
62. Boehm, A. & Vogel, J. The *csgD* mRNA as a hub for signal integration via multiple small RNAs. *Mol. Microbiol.* **84**, 1–5 (2012).
63. Mandin, P. & Guillier, M. Expanding control in bacteria: interplay between small RNAs and transcriptional regulators to control gene expression. *Curr. Opin. Microbiol.* **16**, 125–132 (2013).
64. Mika, F. *et al.* Targeting of *csgD* by the small regulatory RNA RprA links stationary phase, biofilm formation and cell envelope stress in *Escherichia coli*. *Mol. Microbiol.* **84**, 51–65 (2012).
65. Seitz, H. Redefining microRNA targets. *Curr. Biol.* **19**, 870–873 (2009).
66. Figueroa-Bossi, N. *et al.* RNA remodeling by bacterial global regulator CsrA promotes Rho-dependent transcription termination. *Genes Dev.* **28**, 1239–1251 (2014).
67. Jorgensen, M. G., Thomason, M. K., Havelund, J., Valentin-Hansen, P. & Storz, G. Dual function of the McaS small RNA in controlling biofilm formation. *Genes Dev.* **27**, 1132–1145 (2013).
68. Fender, A., Elf, J., Hampel, K., Zimmermann, B. & Wagner, E. G. RNAs actively cycle on the Sm-like protein Hfq. *Genes Dev.* **24**, 2621–2626 (2010).
69. Hussein, R. & Lim, H. N. Disruption of small RNA signaling caused by competition for Hfq. *Proc. Natl Acad. Sci. USA* **108**, 1110–1115 (2011).
70. Moon, K. & Gottesman, S. Competition among Hfq-binding small RNAs in *Escherichia coli*. *Mol. Microbiol.* **82**, 1545–1562 (2011).
71. Fröhlich, K. S., Papenfort, K., Fekete, A. & Vogel, J. A small RNA activates CFA synthase by isoform-specific mRNA stabilization. *EMBO J.* **32**, 2963–2979 (2013).
72. Guillier, M. & Gottesman, S. Remodelling of the *Escherichia coli* outer membrane by two small regulatory RNAs. *Mol. Microbiol.* **59**, 231–247 (2006).
73. Mandin, P. & Gottesman, S. Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. *EMBO J.* **29**, 3094–3107 (2010).
74. Papenfort, K., Espinosa, E., Casadesus, J. & Vogel, J. Small RNA-based feedforward loop with AND-gate logic regulates extrachromosomal DNA transfer in *Salmonella*. *Proc. Natl Acad. Sci. USA* **112**, E4772–E4781 (2015).
75. Thomason, M. K., Fontaine, F., De Lay, N. & Storz, G. A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in *Escherichia coli*. *Mol. Microbiol.* **84**, 17–35 (2012).

Acknowledgements
Research in the laboratories of L.B. and N.F-.B. is funded by the Centre National de la Recherche Scientifique (CNRS) and by the Agence Nationale de la Recherche (grants BLAN07-1_187785 and ANR-3-BSV3-0005).

Competing interests statement
The authors declare no competing interests.