Small Regulatory RNAs in Bacteria

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Intergenic regions of bacteria contain small regulatory ribonucleic acid (srRNA) genes whose transcripts control expression of distal genes. These transcripts, referred to here as srRNAs, primarily act at the level of translation where they bind messenger RNAs (mRNAs). srRNAs can inhibit or activate a target mRNA. Base pairing with mRNAs is imperfect and includes looped out and/or bulged nucleotide positions and noncanonical base pairs as well. The RNA chaparone protein Hfq is involved in many RNA/RNA interactions and ribonucleases, RNase E and RNase III, have been implicated in the destabilization of several target mRNAs. Gene transcription can also be controlled by an srRNA via binding to the RNA polymerase–sigma factor complex and blocking a functional site on the enzyme complex. Many srRNA genes are transcriptionally activated by environmental stress factors and have complex promoter and upstream regulatory sites involved in this activation. The control of outer membrane protein synthesis in response to stress is one major function of bacterial srRNAs.

Advanced article

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Introduction

Numerous small regulatory ribonucleic acid (srRNA) genes have been found in chromosomal intergenic regions of prokaryotes and eukaryotes. Most of these do not encode proteins but produce small RNA transcripts, generally less than 200 nt, that function as regulatory elements. For the most part, these RNAs control gene expression posttranscriptionally by base pairing with target messenger RNAs, but some bind proteins and modulate transcription (Barrandon *et al*., 2008). In the past, srRNA genes have been called noncoding RNA genes. However, as there are two examples where transcripts serve as both a regulator and an mRNA encoding a polypeptide (Wadler and Vanderpool, 2007; Balaban and Novick, 1995; Boisset *et al*., 2007), and the possibility remains that more RNAs with dual roles will be found, a change in terminology is in order. Most srRNA genes respond to environmental stress and/or internal stress signals. srRNA genes were originally found and characterized in bacteria (Mizuno *et al*., 1984; Andersen *et al*., 1987; Andersen *et al*., 1989) but a multitude of these RNAs are now known to be present in eukaryotes (Storz *et al*., 2005; also see microRNA *Nature Reviews* webpage: http://www.nature.com/reviews/focus/microrna/index.html). Prokaryotic srRNAs and the class of eukaryotic RNAs called microRNAs regulate target gene transcripts by similar overall mechanisms, i.e. these RNAs are encoded in genomic loci that are in different chromosomal locations from those which encode target transcripts; they bind target mRNAs and subsequently inhibit gene expression by preventing translation and/or inducing destabilization of the mRNAs. However, the ancillary protein machinery employed in this regulatory process and the evolutionary origins of the regulatory processes are probably very different. Eukaryotes employ intricate protein complexes to process precursor srRNA transcripts and facilitate RNA/RNA binding, but these complexes and transcript processing mechanisms have yet to be found in bacteria, although bacterial RNA-binding proteins are believed to facilitate RNA/RNA duplex formation. Mechanisms associated with regulation by eukaryotic RNAs are covered in the *Encyclopaedia of Life Sciences* by Hannon. In the present article, the molecular and genetic aspects of regulation of gene expression by srRNAs in bacteria are presented. As there is a wealth of information on regulation of outer membrane porin protein synthesis, a part of this treatise will concentrate on this regulation. **See also**: RNA Interference (RNAi) and MicroRNAs

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Bacterial srRNAs

The majority of known bacterial regulatory RNAs are from the Gram-negative bacteria *Escherichia coli* and *Salmonella*. A compilation of experimentally

characterized *E. coli* small RNAs, both regulatory and housekeeping RNAs can be found in Rfam, the Welcome Trust Sanger Institute database of RNA families website (http://www.sanger.ac.uk/Software/Rfam/) (Griffiths *et al.*, 2005). A list of regulatory srRNAs and their targets is provided in **Table 1**. Most srRNAs shown are those transcribed from small RNA genes, which are independent transcriptional units with their own promoters. The number of srRNAs shown is probably on the low side as transcriptional analysis of intergenic regions using microarrays and computational approaches indicate several hundred RNA transcripts in intergenic regions (Tjaden *et al.*, 2002; Saetrom *et al.*, 2005). But many of these have yet to be characterized. By using a combination of assays, including the pyrosequencing technique, 64 RNAs have been detected in *Salmonella typhimurium* (Sittka *et al.*, 2008). The work by Sittka *et al.* represents the most up-to-date evaluation of srRNAs in this organism.

Mechanisms of Regulation

srRNAs regulate mRNA targets by base pairing. They either inhibit translation by binding to the ribosome-binding site (RBS) on the mRNA, or activate the mRNA by unblocking a previously sequestered RBS. **Figure 1** and **Figure 2** depict a generalized outline of the regulation of expression of a target gene by srRNAs. The srRNA and target mRNA transcripts originate from distal parts of the chromosome. In the first example, base pairing between the two RNAs results in shielding of the RBS (**Figure 1**). The pairing is generally imperfect, with bulged and looped

Table 1 List of *E. coli* small regulatory RNAs and target molecules

RNA	Target	Effect of target function
csrB RNA	CsrA protein	Suppression
csrC RNA	CsrA protein	Suppression
cyaR RNA	*ompX* mRNA	Suppression
dicF RNA	*ftsZ* mRNA	Suppression
dsrA RNA	*rpoS* mRNA; *hns* mRNA	Activation
gadY RNA	*gadX* mRNA	Activation
gcvB RNA	*oppA* and *dppA* mRNAs	Suppression
invR RNA	*ompD* mRNA	Suppression
micA RNA	*ompA* mRNA	Suppression
micC RNA	*ompC* mRNA	Suppression
micF RNA	*ompF* mRNA	Suppression
omrA RNA	*ompT* mRNA, *cirA* mRNA, *fecA* mRNA, *fepA* mRNA + 32 other mRNAs	Suppression
omrB RNA	*ompT* mRNA, *cirA* mRNA, *fecA* mRNA, *fepA* mRNA + 14 other mRNAs	Suppression
oxyS RNA	*fhlA* mRNA + > 40 genes indirectly	Suppression
rprA RNA	*rpoS* mRNA	Activation
RseX RNA	*ompA* mRNA, *ompC* mRNA	Suppression
rybB RNA	[a]*ompC* mRNA, *ompD* mRNA + other *omp* mRNAs	Suppression
ryhB RNA	*sodB* mRNA + several other mRNAs	Suppression
rydC RNA	*yejABEF* mRNA	Suppression
ryeB RNA	Unknown	
srgS RNA	*ptsG* mRNA	Suppression
Spot42 RNA	*galK* mRNA of galETKM mRNA	Suppression
sraC RNA	Unknown	–
sraG RNA	Unknown	–
sraH RNA	Unknown	–
sraJ RNA	Unknown	–
sroB RNA	Unknown	–
sroC RNA	Unknown	–
sroD RNA	Unknown	–
sroE RNA	Unknown	–
sroH RNA	Unknown	–
6S RNA	RNA polymerase/σ^{70} complex	Suppression

[a]mRNA binding shown in *Salmonella*.

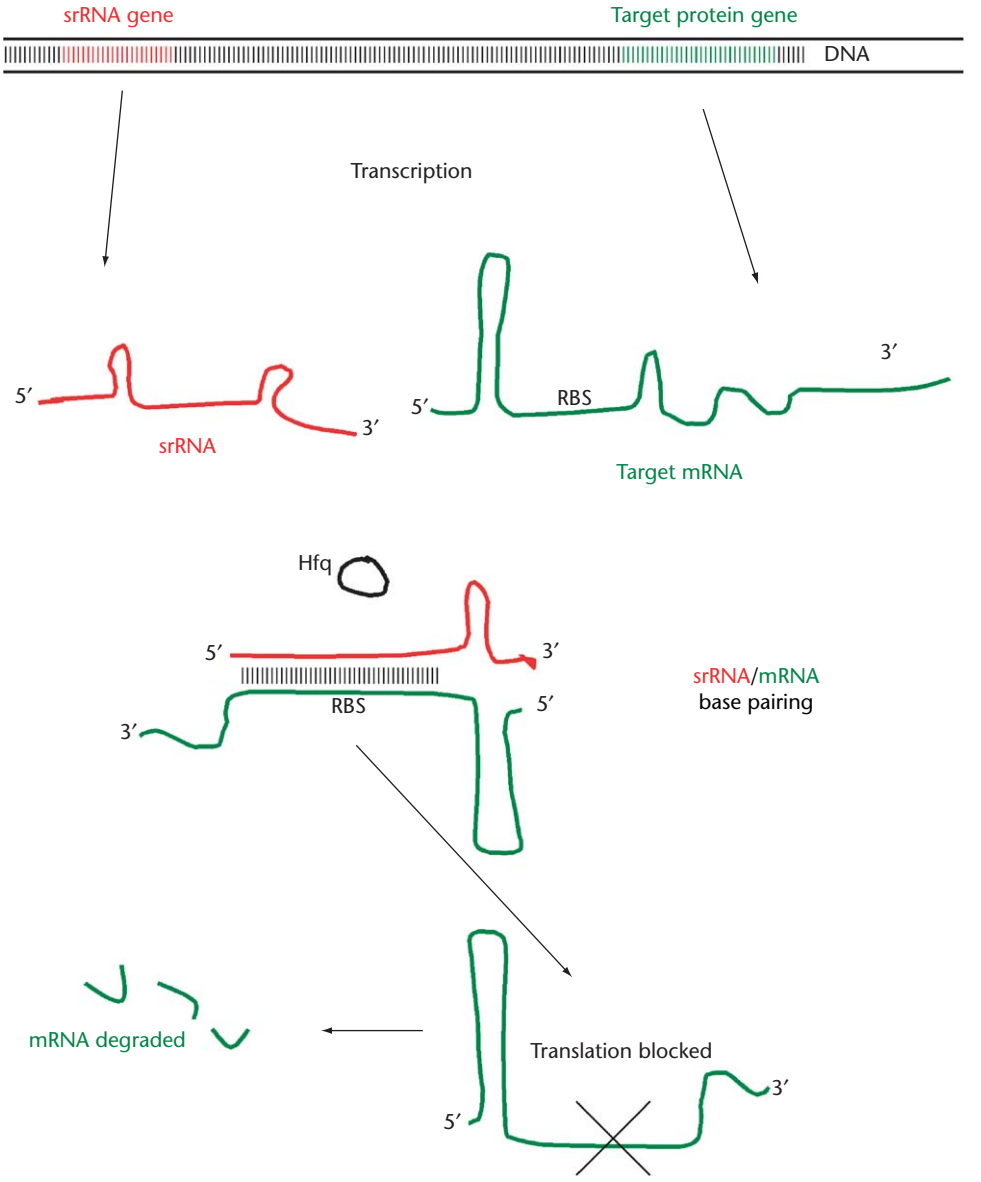

Figure 1 Generalized representation of an srRNA transcript interacting with a target mRNA. Hfq is the RNA-binding protein that facilitates the RNA/RNA interaction. RBS is the ribosome-binding site on the mRNA. Translation is blocked and in many cases the mRNA is degraded.

nucleotide position within the base-paired stem (see later discussion). The RNA chaperone protein Hfq is known to bind to many *E. coli* and *Salmonella* srRNAs (Zhang *et al.*, 2003; Sittka *et al.*, 2008). It is an important factor in regulatory RNA/target RNA binding. The RNA/RNA complex leads to translational inhibition, and usually results in the degradation of the target mRNA. In some cases the srRNA is also degraded. Ribonucleases, RNase E and RNase III, have been implicated in RNA destabilization induced by srRNAs (Urban and Vogel, 2007). RNase E is an endoribonuclease that cleaves in internal single-stranded sequences of mRNAs and fragments the RNA. RNase III is a double-stranded RNA-cleaving enzyme that cleaves both strands. srRNAs can also activate a silent mRNA whose RBS is sequestered by intramolecular base pairing (**Figure 2**). In this model, the target mRNA is activated by the binding of an srRNA to the 5′ side of the mRNA and subsequently altering the mRNA conformation. This results in exposure of the RBS, which is now in a single-stranded form; and this enables the ribosome to bind the RBS and initiate translation from the mRNA.

srRNAs can also inhibit gene expression indirectly by binding a protein crucial to expression of a gene or group of genes. The 6S RNA inhibits transcription from a large number of genes in this fashion, by binding the RNA polymerase factor σ^{70} (Wassarman, 2007).

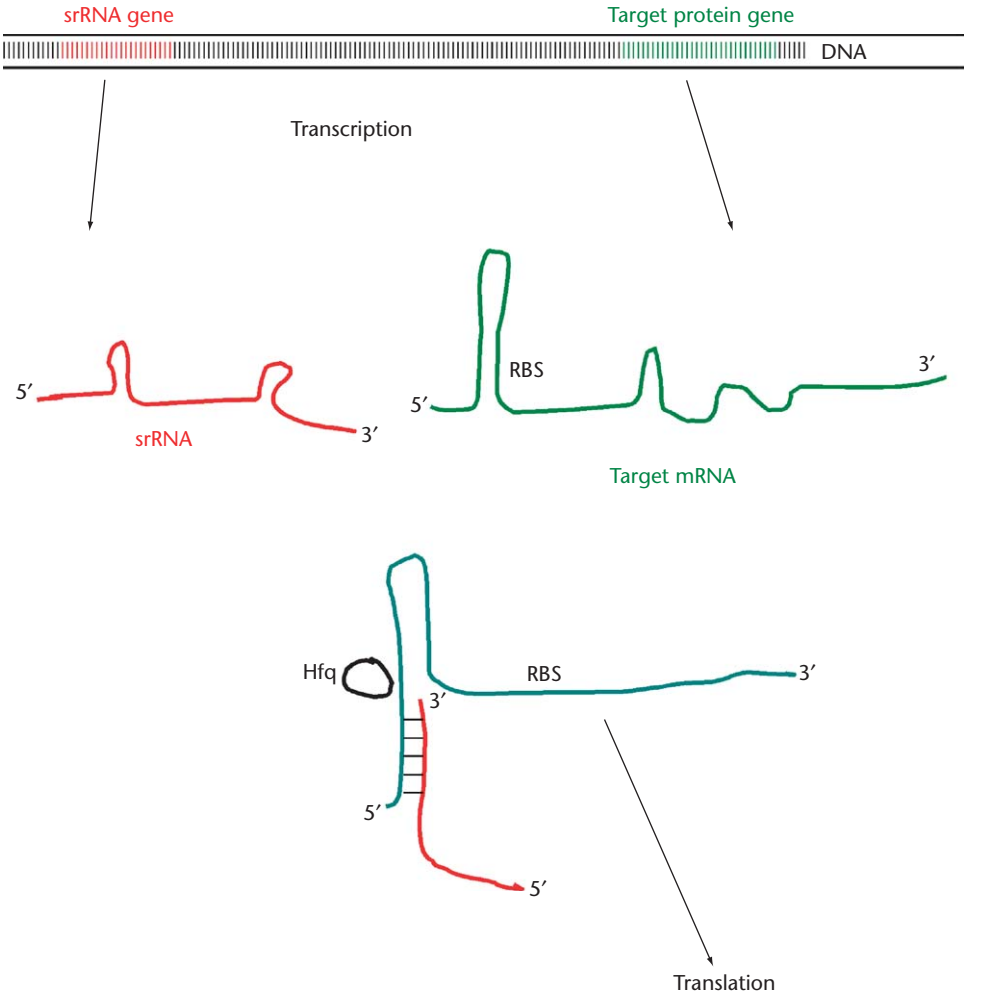

Figure 2 Representation of the activation of translation of an mRNA by srRNA. The RBS is normally sequestered in the base pairing of the 5′ UTR stem loop. When the srRNA is transcribed, it can bind to the 5′ end of the mRNA and produce a conformational change resulting in the exposure of the RBS.

Examples of Regulation by Bacterial srRNAs

We focus on four aspects of regulation:

1. Control of outer membrane protein (Omp) synthesis. A significant number of Omps are downregulated by inhibition of *omp* mRNA translation.
2. Translational control by srRNA via activation of target mRNA, the regulation of *rpoS*, which encodes an RNA polymerase–sigma factor is used as a model.
3. Regulation of a polymerase–sigma factor by 6S RNA. This is the example of inhibition of a protein by an srRNA, rather than inhibition of an mRNA.
4. Dual functions of an srRNA. There are currently two examples of srRNAs serving as both a riboregulator and an mRNA that encodes a protein. The *sgrS* RNA is used as an example.

Control of Porin Gene Expression

Surface proteins are important to the survival of the bacterial cell. Several are regulated by srRNAs in response to environmental conditions. These include the outer membrane porin proteins, which are present in *E. coli* and other Gram-negative bacteria. Some Omps, such as OmpF and OmpC form channels and function to allow passage of small molecules such as nutrients and ions through the outer membrane. They also facilitate the excretion of waste products and help prevent toxins from entering the cell. *ompF* and *ompC* are regulated both transcriptionally and posttranscriptionally, and expression of these porin genes is affected by environmental factors. OmpX is one of the smallest porins. Although its role as an outer membrane is unknown, it may promote adhesion to mammalian cells. OmpA serves to secure the outer membrane to the cell by covalent linkage to the

petidoglycan, the cell wall. In view of the number of Omps that are regulated by srRNAs, a major focus will be on *omp* gene regulation.

micF RNA

The concept that an RNA gene can regulate expression of an unlinked gene at the level of the mRNA was first formulated with analyses of sequences upstream of OmpC gene (*ompC*) in *E. coli* (Mizuno *et al*., 1984). Overexpression of these upstream sequences by multicopy plasmids inhibited expression from the distal gene *ompF*, which encodes OmpF. The regulating sequence was termed *micF* (messenger inhibitory complementary). Definitive evidence for existence of this regulatory RNA gene, its promoter, the RNA transcript, the RNA/RNA base pairing and the gene's functional role was provided in a series of subsequent articles (Andersen *et al*., 1987, 1989; Coyer *et al*., 1990; Andersen and Delihas, 1990; Schmidt *et al*., 1995). Initially, the concept that a small RNA gene can be a gene regulator was met with resistance, albeit the related *cis*-acting antisense RNAs were well known and well characterized at the time (Tomizawa *et al*., 1981). However, we now know that these regulatory genes are numerous both in prokaryotes and eukaryotes (Storz *et al*., 2005).

ompF is regulated transcriptionally by the transcription factor OmpR in response to osmolarity of growth medium (Hall and Silhavy, 1981). However most of the regulation of *ompF* occurs by *micF* at the posttranscriptional level in response to various environmental stress conditions such as oxidative stress, increase in temperature or the presence of weak acids or toxic compounds, and as well as internal stress such as mutations in cell membrane phospholipids or Omps (Delihas and Forst, 2001). *micF* has a complex promoter and has upstream binding sites for four different transcription activators that activate *micF* transcription in response to particular stress factors.

micF RNA is a 93-nt transcript. It is triphosphorylated at its 5′ end and has a ρ-independent transcription termination signal at its 3′ end (Andersen *et al*., 1987). The *micF* gene maps at 21 min on the *E. coli* chromosome and the target *ompF* gene maps at 48 min. *micF* RNA downregulates *ompF* expression by binding to the 5′ end of *ompF* mRNA, blocking the RBS, and thus blocking translation. In addition, *micF* RNA participates in the destabilization of the message. The mechanism of mRNA destabilization may be via processes described for other srRNAs (see later discussion). Hfq is believed to participate in the RNA/RNA interaction, but this has not been shown experimentally.

A model of the RNA/RNA duplex interaction is shown in **Figure 3**. It displays the type of interactions found intramolecularly in RNAs. The *micF*/*ompF* RNA/RNA duplex has looped and bulged positions as well as a G–G noncanonical base pair. These structures may provide for a particular conformation of the duplex, which may be important for protein binding or other functions. **See also**: Base Pairing in RNA: Unusual Patterns; RNA Structural Motifs

micC RNA

Similar to *ompF*, *ompC* is also regulated by an RNA gene, which is termed *micC* (Chen *et al*., 2004). *micC* is found between protein genes *ompN* and *ydbK* in *E. coli*. It encodes a 109-nt transcript that represses *ompC* expression posttranscriptionally. The levels of cellular *micC* RNA found under different environmental growth conditions are opposite to those of *micF* RNA, e.g. when cells are grown in minimal, *micC* RNA levels are high, and those of *micF* RNA are low (Chen *et al*., 2004; Coyer *et al*., 1990). This and other responses to environmental and stress conditions are consistent with the regulation of *ompC* and *ompF*. By RNA/RNA modelling, *micC* RNA is assumed to bind in the 5′ UTR (untranslated region) region of *ompC* mRNA, adjacent to, but not covering the RBS. It is assumed that binding to this region prevents translational initiation. This may be due to steric hindrance, as the proposed *micC* RNA/*ompC* mRNA interaction is next to the RBS, i.e. RNA/RNA pairing shows a stable stem of 16 contiguous Watson–Crick pairs which may impede ribosome binding to the RBS. In addition, the RBS may be shielded due to higher order structure, but the three-dimensional conformation of this RNA/RNA duplex, or for that matter any other srRNA/mRNA duplexes are not known. *micC* RNA has been shown to bind Hfq and that Hfq is essential for *micC* RNA function and repression of *ompC*. It is assumed that Hfq is required for base pairing of *micC* RNA with *ompC* mRNA.

micA RNA

OmpA is an abundant Omp that plays a structural role in securing the outer membrane to the peptidoglycan, the cell wall. It is actively synthesized during logarithmic phase of growth, but continued synthesis may not be needed when cell growth rate diminishes. Consistent with this concept, *ompA* mRNA is unstable as cells enter stationary phase (Nilsson *et al*., 1984) and concomitantly, a 70-nt srRNA termed *micA* RNA accumulates during this phase (Udekwu *et al*., 2005). This RNA is responsible for most of the decrease found in mRNA levels. Similar to the action of *micF* and *micC* RNAs, *micA* RNA base pairs to a segment of the *ompA* mRNA 5′ UTR containing the RBS and prevents ribosome binding. The base pairing and ribosome inhibition have been well characterized by the use of multiple experimental assays, including toe printing and base pair compensatory changes (Udekwu *et al*., 2005). The RNA-binding protein Hfq facilitates RNA/RNA base pairing, and it has been proposed that the endoribonuclease RNase E participates in degradation of the mRNA transcript once ribosome binding is inhibited. A model of the inhibition and degradation of the *ompA* mRNA is shown in **Figure 4**. Although these studies were performed in *E. coli*, *micA* RNA is found in a wide range of

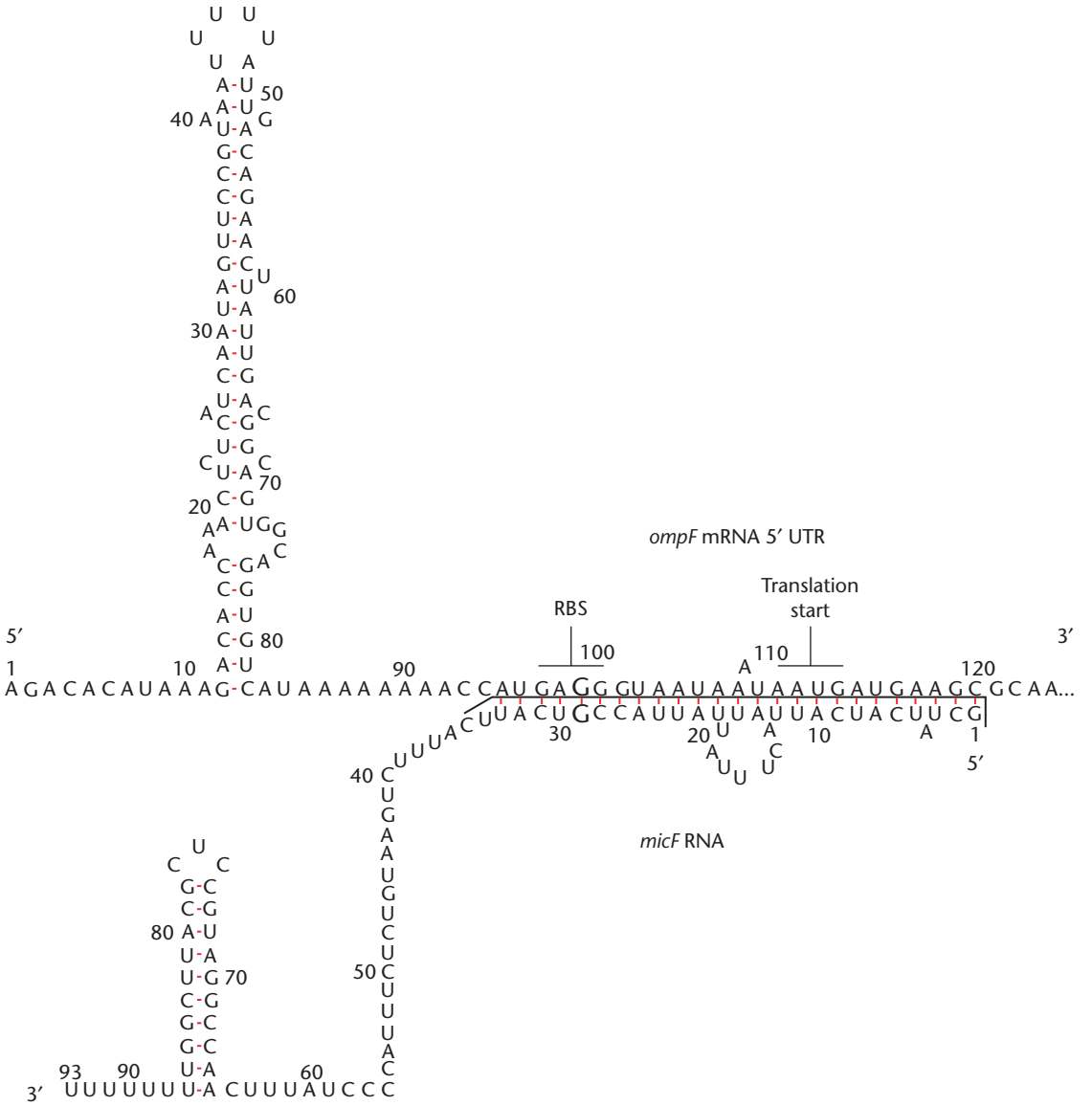

Figure 3 Interaction of the *E. coli micF* RNA with the target *ompF* mRNA. The RNA/RNA duplex structure was determined experimentally. RBS refers to the ribosome-binding site on the mRNA and AUG is the translation start codon. Regulatory and target RNAs from related species of bacteria form similar duplex structures. Modified from Delihas *et al.* (1997).

Gram-negative bacteria, including those in distantly related species such as *Serratia marcescens* and *Photorhabdus luminescens* (Vogel and Papenfort, 2006; Papamichail and Delihas, 2006).

cyaR RNA

OmpX is a small Omp (approximately 172 amino acid, aa) and found highly conserved in a wide range of enterobacteria. It is considered a porin, yet its specific functions are unclear, and some data shows that OmpX is not essential for cell growth (Stoorvogel *et al.*, 1991). Similar to the regulation of expression of *ompF*, *ompX* is also regulated in response to environmental stress (Dupont *et al.*, 2007).

cyaR is an RNA gene present in the intergenic region between protein genes *yegQ* and *orgK* in *E. coli*. The *cyaR* RNA sequence is found highly conserved in a wide range of Gram-negative bacteria, including *Erwinia carotovora* and *P. luminescens* (see Rfam website: http://www.sanger.ac.uk/Software/Rfam/). *cyaR* transcription is activated by cyclic AMP (adenosine monophosphate) (Papenfort *et al.*, 2008). By using the *Salmonella enterica* serovar Typhimurium strain as a model organism, *cyaR* RNA has been shown to repress *ompX* gene expression by base pairing to the RBS sequences, as well as by binding to

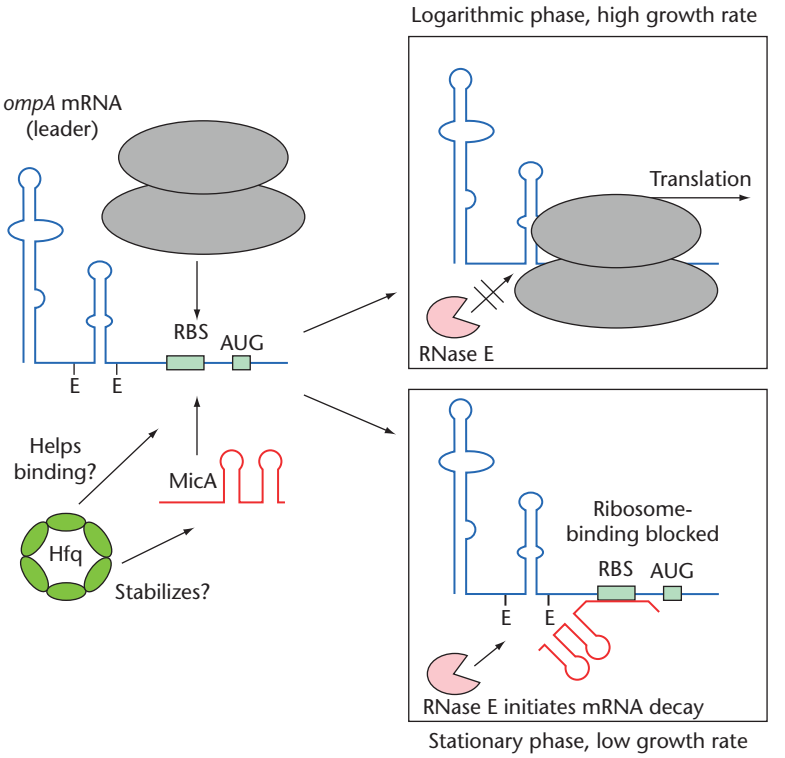

Figure 4 Diagrammatic representation of the regulation of *ompA* RNA by *micA* RNA. Modified from Udekwu *et al.* (2005). Left: *micA* RNA competes for binding to the RBS of *ompA* mRNA. Hfq facilitates RNA/RNA binding. Right: Model of translation from *ompA* mRNA (top) and inhibition and initiation of *ompA* mRNA degradation (bottom). 'E' represents RNase E cleavage sites. Reproduced by permission of Cold Spring Harbor Press.

adjacent sequences of the 5′ UTR of *ompX* mRNA (Papenfort *et al*., 2008). Hfq is believed to be involved in the RNA/RNA interaction based on OmpX accumulations in an *hfq* deletion strain.

cyaR RNA levels are low in exponential phase of growth but its levels are high in early stationary phase. Papenfort *et al*. hypothesize that *cyaR* RNA functions to limit OmpX levels in early stationary phase. The functions of *ompX* in *Salmonella* are not completely understood, but in *E. coli*, the OmpX protein may be involved in glucose uptake. If the same prevails in *Salmonella*, *cyaR* RNA may repress *ompX* expression during entrance to stationary phase when glucose becomes limited. *Salmonella cyaR* RNA is induced in response to other environmental stress factors, and *ompX* may be downregulated when cells are exposed to epithelial cells in the mammalian intestine (Papenfort *et al*., 2008).

omrA and *omrB* RNAs

omrA and *omrB* are tandem RNA genes, but each gene has its own promoter. These RNA genes are found in a wide range of organisms, which include *E. coli, Yesinia pestis* and *Er. carotovora*. In *E. coli*, they are situated between protein genes *ass* and *galR* (Guillier and Gottesman, 2006). The −10 and −35 promoter sequences are well conserved phylogenetically, but the *omrA* and *omrA* promoters differ between themselves. This may allow for independent activation/suppression of expression of these RNA genes. The upstream transcriptional regulatory region from −42 to −110 also shows sequence conservation, which suggests that similar transcription factor(s) may regulate *omrA* and *omrB* in different bacteria. Indeed, the transcription factor OmpR that regulates *ompF, ompC*, *micF* and *micC* expression, also regulates *omrA* and *omrB*.

The *omrA* and *omrA* RNA transcripts are 88 and 82 nt, respectively, and both bind Hfq. Thus they may regulate target mRNAs by base pairing. These srRNAs serve as an important model in that they regulate multiple genes. Microarray and Northern blot analyses appear to show that the RNAs regulate expression from Omp genes *ompT*, *cirA*, *fecA* and *fepA*, and possibly other protein genes. *OmpT* and *cirA* showed the largest changes in mRNA levels due to srRNAs. *fepA* gene expression is suppressed only by *omrA* RNA and not by *ompB* RNA.

RNA/RNA duplex structure modelling indicates that only with *ompT* mRNA is the RBS blocked. This raises questions concerning mechanism of regulation of the other genes and mRNAs.

omrA and *omrB* genes are activated by high osmolarity conditions in the growth media. Thus one role of these RNAs is to repress certain target genes under high osmolarity.

Activation of a Target mRNA by srRNA

Four srRNAs are known to activate translation of target mRNAs: *dsrA*, *rprA*, *rydC* and *gadY* RNAs. *DsrA* and *rprA* RNAs both act on the *rpoS* mRNA. *rydC* RNA appears to interact and activate the polycistronic *yejABEF* mRNA, which encodes the ABC permease (Antal *et al*., 2005). *gadY* RNA base pairs with the 3′ UTR of the target *gadX* mRNA and increases the stability of the mRNA. *gadX* encodes a transcriptional regulator of the acid response genes. Here we concentrate on regulation of *rpoS* expression by the 87-nt *dsrA* RNA as a model for translational activation of target mRNAs.

rpoS encodes the sigma factor σ^s, a subunit of the RNA polymerase, which regulates expression of a large number of genes associated with the response to environmental stress conditions. *rpoS* itself is regulated by several RNA genes; one is *dsrA*. The target *rpoS* RNA normally has its RBS sequestered by surrounding base pairs in the double-stranded stem of the 5′ UTR (**Figure 5a**). Therefore, translation is normally blocked. In addition, there is a double-stranded RNase III cleavage site close to the RBS. RNase III cleaves and destabilizes the untranslated mRNA (Resch *et al*., 2008). When *dsrA* RNA synthesis is induced, the RNA base pairs to a complementary region of the 5′ UTR of *RpoS* mRNA, starting at 97 nt upstream from the AUG translational initial codon (**Figure 5b**). The initial RNA/RNA binding involves a 'kissing interaction' between the loop sequence of one of the *dsrA* RNA stem loops and the mRNA, resulting in unfolding of the stem and subsequently more extensive base pairing between the two RNAs (Lease and Belfort, 2000). The *dsrA* RNA/mRNA interaction creates new RNase III cleavage sites, and frees the RBS for initiation of translation as well (**Figure 5b**). Concomitantly, the mRNA is stabilized, even though upstream sequences are cleaved by RNase III. In this fashion, *dsrA* RNA activates translation of the *rpoS* mRNA.

In addition to regulation of *rpoS*, *dsrA* RNA also interacts and regulates the mRNA encoding the histone-like protein H-NS. H-NS is a global transcription regulator that suppresses expression from multiple genes. With respect to the effect of *dsrA* RNA on phenotypic properties, *E. coli* is more resistant to low pH growth media conditions when *dsrA* is overexpressed (Lease *et al*., 2004). Levels of mRNAs of multiple acid resistance genes increase when *dsrA* RNA is overproduced in the cell by multicopy plasmids. The interaction with *rpoS* and H-NS mRNAs points to *dsrA* RNA as a versatile molecule with very broad effects.

Regulation by Blockage of a Functional Site on a Protein

6S RNA

6S RNA differs from most other regulatory srRNAs in that it does not interact with RNA but interacts with a protein, the RNA polymerase–σ^{70} factor complex. The 6S RNA is also a processed RNA as opposed to most other regulatory RNAs, which are transcripts from independent transcription units. Its genomic sequence is linked to and is co-transcribed with the *ygfA* protein gene, and thus the 6S RNA sequence does not originate from an independent transcriptional unit. 6S RNA binds to the σ^{70} subunit and

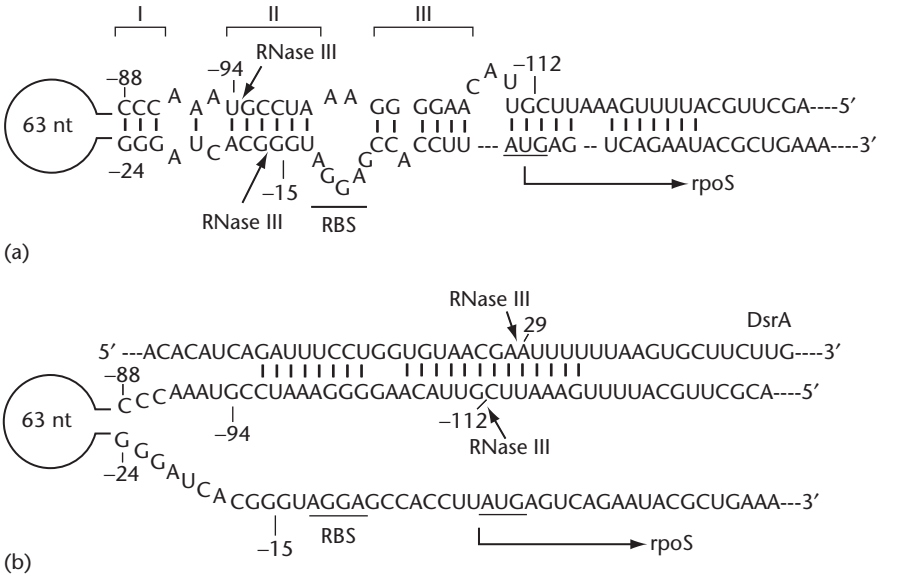

Figure 5 (a) Diagramatic representation of *rpoS* mRNA showing the RBS sequestered by base pairing that is shown in sections I, II and III. Normal RNase III cleavage sites are also shown. (b) The binding of DsrA RNA to the upstream sequence of *rpoS* mRNA exposes the RBS (positions −8 to −12) and creates new RNase III cleavage sites. Modified from Resch *et al.* (2008). Reproduced by permission of the RNA Society.

inhibits RNA polymerase–σ^{70} complex activity (Wassarman and Storz, 2000; Cavanagh *et al*., 2008). 6S RNA plays a regulatory role during stationary phase of bacterial growth. During this phase, 6S RNA levels are high and several hundred genes that are normally transcribed by RNA polymerase–σ^{70} are not expressed. Thus by inactivating the RNA polymerase–σ^{70} complex, 6S RNA is an indirect repressor of gene expression during stationary phase of growth and is an indirect global regulator of gene expression. It has been proposed that 6S RNA binds to the same region of σ^{70} that is important for promoter binding during transcription initiation (Cavanagh *et al*., 2008).

The discovery of 6S RNA is of historical interest. It was first detected in 1967 (Hindley, 1967) and sequenced in 1971 (Brownlee, 1971), albeit its cellular role was unknown at the time. This was one to two decades before it was established that RNAs can act as regulatory molecules. The function of 6S RNA was determined in 2000 (Wassarman and Storz, 2000).

The other known bacterial srRNA believed to bind proteins is CsrB RNA (Babitzke and Romeo, 2007). This RNA interacts with the RNA-binding protein CsrA. CsrA is a global regulatory protein and is involved in regulation of glycogen biosynthesis and glycolysis (Liu *et al*., 1997).

Small Regulatory RNAs with Dual Functions

srgS RNA

The *srgS* gene encodes a transcript that serves as both a regulatory RNA and an mRNA. *srgS* RNA is translated into a small protein, which is also a regulatory molecule. The *srgS* transcript is 220 bp in length. Its 3′-end region base pairs with and inhibits translation from the target mRNA *ptsG* mRNA, which encodes a glucose transporter. The 5′ side of the *srgS* RNA encodes an open reading frame that translates to a 43-amino acid polypeptide termed SgrT. The SgrT protein interferes with glucose 6-phosphate accumulation by preventing glucose uptake (Wadler and Vanderpool, 2007). These interactions are in response to cellular phosphosugar stress. Thus *srgS* is a multifaceted gene that limits synthesis of the glucose transporter protein via RNA regulation, and inhibits the transporter protein function via its translated polypeptide SgrT (Morita and Aiba, 2007).

The other example of an srRNA with dual functions is RNAIII from the Gram-positive organism *Staphylococcus aureus* (Huntzinger *et al*., 2005; Boisset *et al*., 2007). RNAIII suppresses virulence genes and also encodes a virulence protein.

Perspective

During the past 10 years there has been an explosion in the number of bacterial srRNAs that have been discovered. In addition, new findings provide an appreciation for the versatility of small RNAs in terms of mechanisms of regulation. In addition to srRNAs, there are also small RNA transcripts arising from nonautonomous miniature inverted repeat transposable elements (MITEs), which are found in intergenic regions of bacteria (Delihas, 2008). Some MITE transcripts function as regulators of upstream genes. Given that micoarray techniques point to the presence of more small RNA transcripts in bacterial cells compared to those that are presently characterized, future analyses may yield more RNA elements that function as regulators. What is fascinating is that, in addition to known transcripts from srRNA genes, fragments of mRNA 5′ and 3′ UTR sequences have also been detected (Kawano *et al*., 2005). Are these UTR elements functional? There may be more surprises with respect to small RNA functions.

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