Regulatory RNAs and target mRNA decay in prokaryotes

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ABSTRACT

Recent advances in prokaryote genetics have highlighted the important and complex roles of small regulatory RNAs (sRNAs). Although blocking mRNA translation is often the main function of sRNAs, these molecules can also induce the degradation of target mRNAs using a mechanism that drastically differs from eukaryotic RNA interference (RNAi). Whereas RNAi relies on RNase III-like machinery that is specific to double-strand RNAs, sRNA-mediated mRNA degradation in Escherichia coli and Salmonella typhimurium depends on RNase E, a single-strand specific endoribonuclease. Surprisingly, the latest descriptions of sRNA-mediated mRNA degradation in various bacteria suggest a variety of previously unsuspected mechanisms. In this review, we focus on recently characterized mechanisms in which sRNAs can bind to target mRNAs to induce decay. These new mechanisms illustrate how sRNAs and mRNA structures, including riboswitches, act cooperatively with protein partners to initiate the decay of mRNAs. This article is part of a Special Issue entitled: RNA Decay Mechanisms.

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1. Introduction

Post-transcriptional gene regulation mediated by small regulatory RNAs (sRNAs) is commonly found in both prokaryotic and eukaryotic kingdoms. Small RNAs in these systems act to down-regulate target genes by decreasing translation and/or increasing mRNA turnover [1–3]. Eukaryotic microRNAs (miRNA) or small interfering RNAs (siRNA) are assembled into ribonucleoprotein complexes known as RNA-induced silencing complex (RISC) [1,2]. RISCs are composed of a variety of proteins such as RNA-binding proteins, RNA helicases, and nucleases. These characteristics are reminiscent of bacterial sRNAs and RNA-binding protein Hfq, both of which form ribonucleo-protein complexes with the ribonuclease RNase E [4]. Although there are multiple functional similarities between eukaryotic and prokaryotic processes, this review will focus on prokaryotic systems of mRNA decay, notably in Escherichia coli.

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In bacteria, sRNAs are usually non-coding and smaller than 300 nucleotides. At present, ~100 sRNAs have been identified, located either on *E. coli* plasmid or its chromosome [3,5]. Antisense sRNAs, which act by base-pairing with mRNA to inhibit translation of their targets, represent the major class of sRNAs in bacteria. This group can be subdivided as true antisense RNAs or *cis*-encoded sRNAs, synthesized from the strand complementary to the mRNA they regulate, or *trans*-encoded sRNAs, synthesized at a different genomic location. The latter type of sRNA possesses limited complementarity with mRNA targets (about 7 to 12 bases) that enables *trans*-encoded sRNAs to modulate the activity and stability of multiple mRNAs [3]. The segment of contiguous base-pairing is called “seed region” by comparison to eukaryotic microRNA system [6]. Basically, base-pairing between sRNA and mRNA targets can lead to the activation or inhibition of mRNA translation (RNAIII, [7]), mRNA stabilization (GadY, [8]) or mRNA degradation (RyhB, [9]).

### 2. sRNAs as mRNA translation modulators

Most sRNAs characterized to date block translation by direct binding to the ribosome-binding site (RBS) in the 5′-UTR (UnTranslated Region) of target mRNAs (Fig. 1A). Basically, sRNA sequester and mask the RBS through interactions involving short regions (7–12 bases) of imperfect complementarity, to prevent 30S ribosome binding and translation initiation.

In contrast, some sRNAs activate translation by binding to the 5′-untranslated region (5′-UTR) of the target mRNA. Usually, these target mRNAs harbor an intrinsic secondary structure in the 5′-UTR that inhibits ribosome binding. Thus, when a sRNA binds to the inhibitory sequence in the 5′-UTR, the RBS becomes available, allowing initiation of translation (Fig. 1D). For instance, this regulation mechanism was shown for both DsrA and RyhB sRNAs, which stimulate RpoS [10] and shiA translation initiation in *E. coli* [11,12], respectively. Another example is the activation of hla mRNA translation by the 5′-end of RNAIII sRNA in *Staphylococcus aureus* [7]. One can speculate that the number of sRNAs activating mRNA translation is most likely underestimated as the effect on targeted mRNAs is often too subtle to be detected by microarrays or Northern blots. Remarkably, whether the target mRNAs are activated or repressed by sRNAs, they require the RNA chaperone Hfq for the full extent of regulation at least in *E. coli* and *Salmonella*.

### 3. The RNA chaperone Hfq and mRNA translation repression

In *E. coli*, most sRNAs that bind to mRNAs depend on the 11 kDa RNA chaperone Hfq. *In vivo*, Hfq monomers assemble to form hexamers and dodecamers, which stabilize sRNAs and modulate base-pairing with target mRNAs [13–15]. Several studies have shown similarities in both protein sequence and structure between bacterial Hfq and eukaryotic Sm proteins, which bind small nucleolar RNAs and are components of the spliceosome in eukaryotes [16]. Recently, a number of studies, using Hfq as bait, have identified a few dozen sRNAs bound to this chaperone protein in *E. coli* [17,18] and *Salmonella* [19–21]. Based on the fact that Hfq binds to so many sRNAs, it is likely that Hfq plays a key role in mRNA translation repression in prokaryotes.

![Fig. 1. Small RNA-based regulatory mechanisms of mRNA expression.](http://dx.doi.org/10.1016/j.bbagen.2013.02.013)
sRNAs, it has become the focus of intensive research aimed at a better understanding of its cellular role. The chaperone Hfq likely helps binding through remodeling RNA structures and by increasing local concentrations of the sRNA and target mRNA [13–15,22].

A recent report has provided evidence that Hfq is recruited by sRNA Spot24 and directly represses mRNA translation [23]. According to this novel mechanism, Spot24 is exclusively involved in Hfq recruitment and does not contribute directly to mRNA regulation. This is the first example of a “role reversal” between a sRNA and Hfq where Hfq is directly involved in the translational repression of the target mRNA and where the sRNA acts only as a recruitment factor.

Furthermore, several groups have shown that Hfq can also bind target mRNAs such as sodB, iscS, and sdhc even in the absence of sRNA [22,24]. This direct binding on target mRNAs suggests that Hfq may help to recruit the ribonucleoprotein silencing complex on specific mRNAs.

4. Hfq antagonizes RNase E activity

Hfq is also a key player in the modulation of mRNA stability. In fact, Hfq can protect transcripts against ribonuclease E (RNase E) attacks due to coincident Hfq binding sites and RNase E cleavage sites on mRNA (AU-rich single-strand regions) [4]. RNase E is a single-strand-specific endoribonuclease that initiates the decay of many mRNAs in E. coli. Subsequently to RNase E-dependent cleavage, the resulting intermediate products are degraded by endo- and exoribonucleases (e.g. polynucleotide phosphorylase (PNPase), RNase II, and RNase R). RNase E is part of a multipartic complex called RNA degradosome that is composed of a 3′-exoribonuclease (polynucleotide phosphorylase or PNPase), a DEAD-box RNA helicase (RHb), a glycolytic enzyme (enolase), and other proteins, depending on physiological conditions [25,26].

5. sRNA-mediated mRNA decay

A new family of sRNAs with a more complex mechanism of action was first characterized ten years ago [9]. This group of sRNAs, which quickly became a paradigm in sRNA mechanism, was shown to be base-pair with target mRNAs to trigger their degradation by RNase E (Fig. 1B and C). Incidentally, RNA-binding Hfq was also shown to be involved in the recruitment of RNase E and sRNA-mediated mRNA decay [30,31]. The first example of sRNA involved in sRNA-mediated mRNA degradation was RyhB, which coordinates bacterial response to iron starvation. The sRNA RyhB is essential for the iron-sparing response that redistributes scarce amounts of intracellular iron and allows growth during severe iron-limitation [32–34]. RyhB induces the rapid degradation (within 10 min) of at least 18 mRNAs, which all encode iron-using proteins, to redirect iron to essential regions of the cell [9,35]. A similar mechanism of fast mRNA decay was recently confirmed for additional sRNAs such as SgrS [36], MicC [37], GcvB [38], OmrA and OmrB [39–41], and RybB [42].

The rapidity of sRNA-mediated mRNA degradation suggests that target mRNAs have to be silenced as soon as possible under conditions where RyhB is expressed (low iron). Remarkably, Morita et al. [43] showed that translational repression occurs in the absence of sRNA-mediated mRNA degradation for the SgrS/ptsG and RyhB/sodB duplexes. Thus, in most cases, translational repression is sufficient for gene silencing. One possible physiological role of mRNA degradation mediated by sRNAs is to make gene silencing irreversible. It has been observed that during the process of sRNA-mediated mRNA degradation, the sRNA is degraded stochiometrically together with its target [4,9,44]. However, recent data suggest that the stoichiometric cleavage of sRNA and target mRNA may not be concomitant in the case of all sRNAs. Indeed, whereas sRNA MicC in Salmonella induces the degradation of ompD mRNA, MicC itself becomes degraded in a sequential manner, possibly when it is liberated from the degraded target [45].

The mechanism by which sRNAs/Hfq association leads to the degradation of target mRNAs by RNase E remains unclear. Two pathways can be proposed to explain mRNA destabilization. According to one pathway, mRNAs may become more sensitive to RNase E attacks after base-pairing with sRNA, as a result of the loss of protection conferred by translating ribosomes (Fig. 1B, passive nucleolytic repression) [46]. According to the other pathway, recruitment of RNase E on the target mRNA triggers formation of a sRNA/Hfq/RNase E complex that favors RNase E attacks; this complex then becomes a specialized RNA decay machine (Fig. 1C, active nucleolytic repression). In fact, in some cases such as MicC ompD duplex, it cannot be assumed that mRNA decay is due to an indirect effect of mRNA translation inhibition. The sRNA MicC has been first identified as a repressor of the major porin OmpC synthesis in E. coli. MicC blocks ompC translation by competing with ribosome binding [47]. In Salmonella typhimurium, MicC sRNA also pairs within the coding sequence (CDS) of ompD mRNA [37]. Due to the fact that MicC binds the ompD mRNA far downstream of the RBS, MicC is unable to directly block ompD translation initiation. In order to abrogate OmpD synthesis, MicC has to induce a specific RNase E-dependent cleavage in the CDS of ompD [37]. In S. typhimurium, a similar mechanism is observed in lpxR mRNA regulation by MicF sRNA [48]. Furthermore, a recent study [49] has shown that the pairing of RyhB to target mRNA sodB initiates mRNA degradation even in the absence of translation of mRNA target. In fact, even if RyhB pairs at RBS, RyhB induces mRNA cleavage at a distal site located hundreds of nucleotides downstream, in the coding sequence of sodB mRNA. Despite these observations, the mechanism by which sRNAs induce distal mRNA cleavage remains obscure. To expand on these findings, Bandrya et al. [45] have suggested that sRNA/mRNA duplexes could actively stimulate RNase E attacks, contrasting with a simple and passive model of RNase E recruitment.

RNase E is known to be involved in two mRNA decay pathways: a first and direct pathway allowing cleavage of triphosphorylated primary transcripts (described above) and a 5′-end-dependent mRNA degradation pathway which requires prior conversion of the 5′-triphosphate extremity to a 5′-monophosphate by the RNA pyrophosphohydrolase RppH, in a manner similar to decapping of mRNAs in eukaryotes [50]. In fact, RNase E favors binding on a monophosphorylated 5′-end (5′-P end), which can bind in a discrete pocket on the surface of RNase E, facilitating mRNA cleavage at a downstream location by the RNase E active site [51–54]. Bandrya et al. [45] showed that the interaction of the 5′-P end of sRNA with a sensor pocket of RNase E favors closure of the catalytic domain to enhance nucleolytic activity (Fig. 1C). Evidence for this mechanism was obtained by studying artificial RNA duplexes and naturally occurring MicC ompD duplexes. In the specific case of MicC ompD, the sRNA/mRNA/Hfq complex not only recruits RNase E but also directly activates mRNA cleavage. Therefore, it can be suggested that an initial cleavage could induce RNase E activity by generation of a 5′-P end sRNA.

6. RNase III can replace RNase E

RNase E is not the only RNase involved in mRNA decay. Other RNases directly process and degrade mRNA transcripts like RNase III, which cleaves double-stranded RNA [56]. For example, S. aureus sRNA RNAIII is involved in the control of virulence by regulating several mRNAs encoding exotoxins and exoproteases [57]. RNAIII base-pairs with a couple of mRNA target independently of Hfq and induces degradation by RNase III instead of RNase E [58]. A same mechanism is observed for tisAB mRNA translational repression by IstR-I sRNA [59].
In *S. typhimurium*, RNase III has also been shown to regulate MicA, a sRNA involved in porin control, in a target-coupled way [60]. Of note, RNase E is responsible for the control of free MicA levels in the cell.

7. Modulation of RNA stability is a widespread regulatory mechanism for sRNAs

The sRNA GadY is a member of the cis-encoded RNA class. GadY positively regulates the levels of gadW and gadX mRNA that are involved in response to acid stress [61]. Base-pairing of GadY with the intergenic region of the *gadX-gadW* mRNA results in targeted cleavage within the region of complementarity and in the stabilization of each transcript [62–64]. In *E. coli*, the activity of the pleiotropic/global regulator CsrA (carbon storage regulator) is regulated by two sRNAs CsrB and CsrC, which act by sequestering multiple CsrA dimers [65,66]. Suzuki et al. [67] have published that CsrB and CsrC degradation is mediated by a specific factor, termed CsrD. CsrD is not a nuclelease but targets CsrB and CsrC for degradation by RNase E.

Whereas the RNA binding protein Hfq is frequently described as being involved in sRNA/mRNA duplex stabilization, it also stabilizes sRNA that acts by sequestration of regulatory proteins. For example, the Rsm sRNA family in *Pseudomonas aeruginosa*, which is homologous to the Csr sRNA family in *E. coli* (for reviews: [68,69]). Sonnleitner et al. [70] have shown that in *P. aeruginosa* RsmY sRNA is specifically stabilized by Hfq protein. Hfq could protect RsmY against RNase E attack by masking the cleavage site of the enzyme [71].

8. Small RNAs and the termination factor Rho

A Rho-dependent mechanism of transcriptional termination by trans-acting sRNAs has recently been proposed [72]. In bacteria, there are two types of transcriptional termination, Rho-dependent and Rho-independent termination. In the case of Rho-independent termination, the mRNA generally presents a stem–loop structure followed by a poly-uracil sequence, which facilitates dissociation of RNA polymerase from the DNA template. In this connection, Rho-independent transcription termination is a frequent mechanism used by riboswitches [73]. In the case of Rho-dependent termination, the transcription termination factor Rho is assumed to sequentially bind newly synthesized RNA through Rho utilization (rut) sites, to move in an ATP-dependent manner, towards the RNA 3′-end and to reach the transcription elongation complex in order to detach it from template DNA [74]. In *Salmonella*, Bossi et al. [72] have reported that ChiX sRNA indirectly exposes internal rut sites normally hidden by translating ribosomes by inhibition of *chiPQ* mRNA translation. In fact, ChiX down-regulates the distal portion of the bicistrionic *chiPQ* operon by inducing early Rho-dependent transcription termination (Fig. 2). Recently, Rabhi et al. [75] have shown that, in *E. coli*, Hfq is able to bind Rho and to interfere with its activity. Thus, a Rho-dependent transcriptional termination mediated by a sRNA or the sRNA-Hfq complex, may be involved in the regulation of other target mRNAs.

9. Riboswitches

Riboswitches are RNA structures located within the 5′-UTR of mRNAs that regulate gene expression at the level of transcription, translation or splicing [76,77]. These RNA structures can change conformation by directly binding intracellular metabolites (e.g. vitamins or amino acids). Depending on the concentration of the metabolite, riboswitches will adopt either activating (ON) or repressing (OFF) conformations (Fig. 3A). Whereas riboswitches normally regulate in cis, recent results suggest that at least one riboswitch can also regulate in trans, similarly to sRNAs [78]. In this case, the riboswitch functions either in cis, as a conventional riboswitch, or in trans, as a sRNA. The sRNA is processed from the longer transcript by an unknown mechanism.

10. Riboswitches as targets for RNase E-based mRNA decay

Although every riboswitch is known, to date, to regulate either transcription elongation, translation initiation or splicing, it was recently shown that the *E. coli* lysC riboswitch controls both translation initiation and mRNA decay (Fig. 3B). When bound to the amino acid lysine, the *lysC* riboswitch adopts the OFF conformation, which simultaneously blocks translation initiation and rapidly induces RNase E degradation [79]. This rapid degradation suggests an active recruitment mechanism whereby the OFF structure is recognized by RNase E, consistent with an active nucleolytic repression. Because of the unique mechanism of *lysC* riboswitch, it is possible that the riboswitch structure is recognized by RNase E. In contrast to sRNA-mediated mRNA degradation, the nucleolytic cleavage in *lysC* riboswitch does not require the RNA chaperone Hfq [79]. However, the observed regulation of *lysC* is so rapid that it suggests the existence of a highly sophisticated mechanism [79]. Altogether, these studies revealed interesting features of riboswitch-controlled mRNA turnover. A key feature that remains to be elucidated is whether a riboswitch can control gene expression strictly by relying on the modulation of an RNase cleavage site. If this were the case, it would bring additional evidence that a RNA-based world may have evolved to protein-based life.

11. Conclusions

An increasing body of evidence supports the interpretation that, in many organisms including humans, mice, yeasts, and bacteria, transcription does not always map to genes [80–82]. In fact, it has become clearer that transcripts originate throughout the whole genome, including regions previously thought to be silent. This “pervasive” transcription also implies extensive post-transcriptional RNA regulation and processing, as suggested recently in bacteria [81]. Therefore, it is very likely that sRNAs play an even larger role than previously described.
acknowledged in the regulation of gene expression. We believe that this new level of regulation may explain the observed lack of correlation between mRNA and protein levels [83].

12. Uncited references

[27-29,55].

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