MINIREVIEW

Bacterial Small RNA-based Negative Regulation: Hfq and Its Accomplices[*]

Nicholas De Lay[1], **Daniel J. Schu**[1], and **Susan Gottesman**[2]

From the Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

A large group of bacterial small regulatory RNAs (sRNAs) use the Hfq chaperone to mediate pairing with and regulation of mRNAs. Recent findings help to clarify how Hfq acts and highlight the role of the endonuclease RNase E and its associated proteins (the degradosome) in negative regulation by these sRNAs. sRNAs frequently uncouple transcription and translation by blocking ribosome access to the mRNA, allowing other proteins access to the mRNA. As more examples of sRNA-mediated regulation are studied, more variations on how Hfq, RNase E, and other proteins collaborate to bring about sRNA-based regulation are being found.

Post-transcriptional Regulation by Small Noncoding RNAs in Bacteria

The idea that RNAs could function as regulators of gene expression has been around since the earliest studies of gene regulation. In their seminal paper entitled "Genetic Regulatory Mechanisms in the Synthesis of Proteins", Jacob and Monod originally hypothesized, "The specific 'repressor' (RNA?), acting with a given operator, is synthesized by a regulator gene" (1). Although the repressor in the case of the *lac* operon turned out to be the Lac repressor protein, the later discovery of small RNA (sRNA)[3] regulators confirmed their original hypothesis.

Currently, examples of this form of gene regulation are widespread among organisms. Here, we will focus on pairing sRNAs in bacteria and, specifically, those that are often termed *trans*-encoding sRNAs. These RNAs are expressed from the DNA in *trans*, *i.e.* the sRNA genes are far from the genes encoding their mRNA target(s) and have limited complementarity with their target mRNAs. These bacterial sRNAs typically range in length from ~50 to 300 nucleotides. Many of these sRNAs are highly expressed when cells are undergoing some type of stress (for instance, oxidative stress, sugar phosphate accumulation, or nutrient starvation). The sRNAs base pair with their mRNA targets, leading to a variety of outcomes. Base pairing can lead to stabilization and/or translational activation of an mRNA target. Usually, activation occurs by base pairing within the

5′-UTR, changing folding of the 5′-UTR to allow entry of the ribosome and translation to occur (reviewed in Refs. 2 and 3). Another mode of action by sRNAs ultimately leads to translational repression and/or degradation of an mRNA target. In the majority of characterized cases, an sRNA base pairs at or around the ribosome-binding site (RBS) of an mRNA target. This leads to inhibition of translational initiation and, in most cases, the subsequent destabilization of the target. Negative regulation can also occur in other ways, as discussed below. Degradation of the mRNA target reinforces the translational repression and makes it irreversible.

In many bacteria, an RNA chaperone, Hfq, is required for efficient base pairing between an sRNA and its target mRNA (reviewed in Ref. 4). In this minireview, we will focus on recent advances in understanding sRNA-mediated negative gene regulation in *Escherichia coli* and *Salmonella enterica*. More specifically, we will focus on the variety of pathways that lead to sRNA-mediated degradation of specific mRNA targets and how Hfq engages the various proteins associated with these pathways.

Hfq and Its Role in sRNA-mediated Regulation

Originally identified in *E. coli* as a host factor required for the efficient replication of the RNA bacteriophage Qβ (5), Hfq is now understood to function as an RNA chaperone within bacterial cells. Hfq has been characterized as a member of the conserved RNA-binding Lsm (like-Sm)/Sm-like protein family found in eukaryotes, bacteria, and archaea. In eukaryotes, the Sm and Lsm proteins have been implicated in many functions, including roles in mRNA splicing, RNA decapping, and RNA stabilization (reviewed in Refs. 6 and 7). This protein family is characterized as forming a ring-like multimeric complex that binds RNA. The eukaryotic Sm proteins generally form heteromultimeric rings, whereas the bacterial Hfq proteins characterized thus far are homomultimeric.

In recent years, Hfq has gained much attention after it was shown to play a critical role in sRNA-mediated gene regulation. It is required for efficient stabilization and annealing of sRNAs to their mRNA targets (reviewed in Refs. 7 and 8). Homologs have been found in >50% of sequenced bacteria (9). Although the mechanism by which Hfq facilitates these interactions remains unclear, crystal structures in complex with RNA molecules and *in vitro* analyses have shed some light on its multiple RNA-binding surfaces. The *Staphylococcus aureus* Hfq crystal structure in complex with a uridine-rich aptamer revealed that RNA winds around the central cavity of the ring structure on one face of Hfq termed the proximal face (Fig. 1A) (10). A crystal structure of *E. coli* Hfq in complex with a poly(A) aptamer demonstrated that the face opposite to that where poly(U) binds, termed the distal face, preferentially binds adenine-rich sequences (11).

In vitro studies have confirmed that mutants in the proximal face disrupt binding of uridine-rich sequences (12). The proximal face may be the site for initial sRNA interactions. One basis for this interaction may be the Rho-independent terminators

[1] Both authors contributed equally to this work.
[2] To whom correspondence should be addressed. E-mail: gottesms@helix.nih.gov.
[3] The abbreviations used are: sRNA, small RNA; RBS, ribosome-binding site; PNPase, polynucleotide phosphorylase; ARRBD, arginine-rich RNA-binding domain; miRNA, microRNA; RISC, RNA-induced silencing complex.
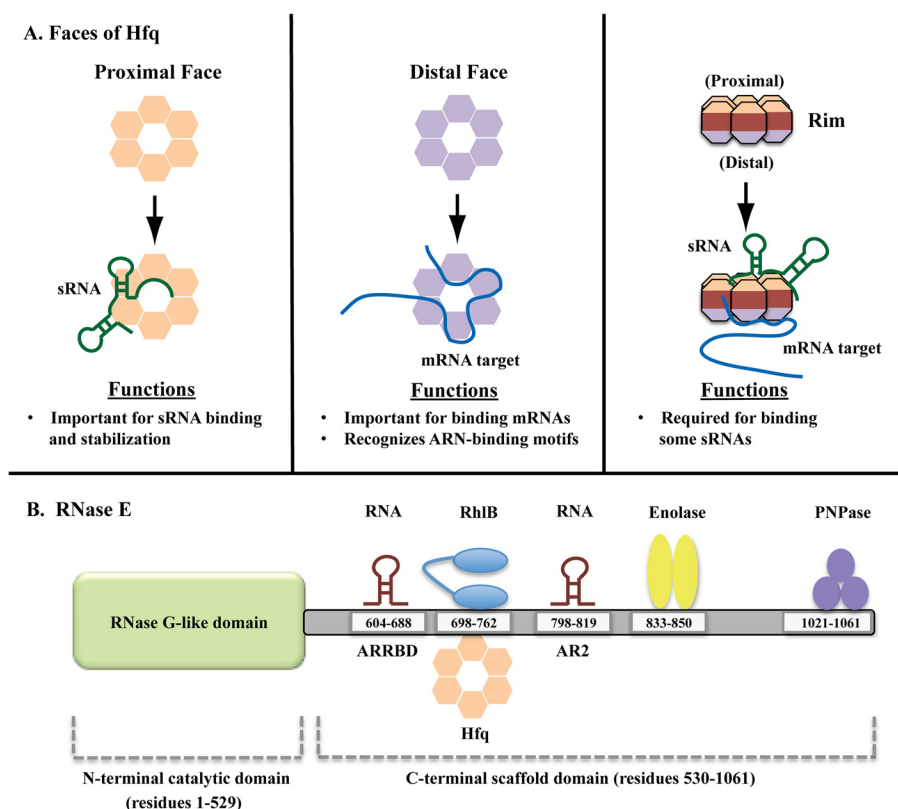
FIGURE 1. **Important protein players in sRNA-mediated gene repression.** *A*, faces of Hfq for RNA interactions, illustrating the proximal face of Hfq (*orange*) bound to sRNA (*green*), the distal face of Hfq (*purple*) bound to an mRNA target (*blue*), and the rim of Hfq (*red*), shown to have interactions with some sRNAs. The currently understood function of each face in sRNA-mediated regulation is summarized at the bottom of each panel. *B*, organization of the RNA degradosome in *E. coli*. The endonuclease RNase E consists of two domains. The N-terminal domain (residues 1–529) resembles RNase G and contains the catalytic site of the enzyme. The C-terminal domain (residues 530–1061) serves as a scaffold domain with which the other components of the degradosome associate to form an active complex. These components and the residues at which they have been shown to interact with RNase E are shown. Interactions of Hfq with RNase E regions closer to the C terminus have also been suggested. See text for references.

with polyuridine tails characteristic of these sRNAs. It has been demonstrated *in vitro* that Hfq has a strong affinity for stem-loops followed by a run of uridines (13–15). However, internal binding sites for Hfq have also been identified in many sRNAs, including OxyS (16), DsrA (17), RyhB (18), and RybB (19). A recent study on SgrS (15) further characterized this internal binding motif; in addition to the Rho-independent terminator, SgrS also required an internal U-rich sequence flanked by a stem-loop for stable binding to Hfq and subsequent sRNA-mediated regulation. The study found that this motif is present in a large portion of the currently characterized sRNAs, suggesting more extensive interactions between Hfq and sRNAs. Recent tests with mutant Hfq proteins indicated that these interactions are abrogated or weakened upon mutation of proximal site residues; these same mutations lead to reduced sRNA accumulation *in vivo*, consistent with loss of tight binding to the sRNAs (20).

Studies on the distal face of Hfq have further characterized it as a site for poly(A) binding and, more specifically, for mRNA interactions (12, 21). The distal face has a strong affinity for mRNAs containing an ARN-binding motif, in which R is purine and N is any base (11). The requirement for an ARN motif for Hfq binding and subsequent sRNA-mediated gene regulation was first reported with the DsrA mRNA target *rpoS* (22). Later studies confirmed the importance of this motif and its role in sRNA regulation (23–25). In addition, mutations in the distal

face binding residues disrupt positive regulation and some negative regulation (20).

Recently, a third site for interaction has been characterized, termed the lateral surface or the rim of the Hfq ring. This surface harbors a positively conserved patch that has been implicated in sRNA binding. It has been suggested that this third interaction site may play an important role in the stepwise association with sRNAs that eventually leads to mRNA binding and dissociation from Hfq (21),[4] and this model is supported by *in vivo* studies of regulation in rim mutants (20).

As noted above, the mechanism by which Hfq facilitates sRNA-mRNA interactions remains unclear. One current hypothesis for the role of Hfq in the cell is to increase the local concentration of the RNAs and thereby increase the likelihood of base pairing. This requires that a given Hfq hexamer bind the appropriate pair of sRNA and mRNA.

Alternatively (or in addition), Hfq may facilitate base pairing by restructuring the RNAs to take on more favorable conformations for interaction (7, 27). Both (or yet other models) could apply in different cases. Regardless, further experiments will be required to elucidate the mechanism by which Hfq facilitates these RNA-RNA interactions.

Although Hfq is an abundant protein, recent studies suggest that there is competition for binding to Hfq (28–30). Each Hfq

---

[4] S. Panja and S. A. Woodson, manuscript in preparation.

hexamer appears to bind one sRNA and one mRNA at a time (31).

Introduction to the Degradosome and Its Role in sRNA Regulation

Studies of proteins that facilitate the role of Hfq in sRNA-dependent negative regulation have focused on RNase E and its role in degradation of target mRNAs (32–35). The N terminus of RNase E encodes an essential endonuclease. RNase E cuts RNAs at single-stranded regions and is important for both mRNA degradation and processing of some structured RNAs.

The C terminus of RNase E acts as a scaffold for the binding of several proteins, including the RNA helicase RhlB, the 3′–5′-exoribonuclease polynucleotide phosphorylase (PNPase), and the glycolytic enzyme enolase (36). The resulting complex of proteins is referred to as the degradosome. RNase E itself is active as a tetramer, and thus, it can be imagined that different subunits of the tetramer may interact with different auxiliary proteins. After endonucleolytic cleavage, PNPase helps to degrade RNAs further by its exonucleolytic activity, and the RhlB helicase is believed to help PNPase deal with secondary structures in RNA targets (reviewed in Ref. 37). Thus, these three proteins provide a machine for the stepwise degradation of RNAs. The role of enolase in this complex is not understood. In addition, the C-terminal scaffold domain has two arginine-rich domains involved in RNA binding (termed ARRBD and AR2) (Fig. 1*B*) (reviewed in Ref. 38).

RNase E was first shown by Massé *et al.* (39) to be important for the coupled degradation of RyhB and its target *sodB* mRNA; it is also necessary for the rapid degradation of RyhB that occurs in the absence of Hfq. Either heat inactivation of a temperature-sensitive RNase E or replacement of *rne* with an allele that expresses RNase E lacking the C-terminal scaffold domain (Fig. 1*B*) inhibits degradation of *sodB* (39). Subsequent studies have shown that RNase E catalyzes degradation of other target mRNAs after sRNA pairing and that the C-terminal scaffold domain of RNase E is required for degradation of these mRNAs (35, 40–42). In addition, Hfq has been found to interact with the scaffold domain of RNase E (Fig. 1*B*; discussed below) (35).

Multiple Pathways for Negative Regulation

Through bioinformatics and experimental approaches, many Hfq-binding sRNAs were identified in *E. coli* and *S. enterica* serovar Typhimurium. One particularly powerful approach has been to isolate Hfq from cells and analyze both the bound sRNAs and mRNAs that are presumably targets for these sRNAs (43–45). Although computational approaches to match sRNAs with their targets are available, these programs still miss many targets and return many false positives. Target mRNAs have been identified for many of these sRNAs by two general experimental approaches. One is to monitor the change in abundance or the turnover of the target mRNA; this approach has been used to identify targets in microarray experiments following short-term overexpression of the sRNA or after expression of the sRNA in response to the natural inducing signal (46, 47). The second approach has been to create a translational fusion of GFP or *lacZ* to a gene of interest and to mon-

itor the effect of deleting or overexpressing a set of sRNAs on this translational fusion (see, for instance, Refs. 48 and 49). Both of these approaches have identified mRNA targets that are negatively regulated by sRNAs. Characterization of the mechanism by which the sRNA leads to the down-regulation of these target mRNAs has revealed that there are multiple pathways for sRNAs to cause degradation of their target mRNAs.

Pairing of the sRNA with a target mRNA can result in translational inhibition by blocking the RBS or ribosome-loading site (Fig. 2*B*). Even binding of an sRNA early within the first few codons of an mRNA can block translation (50). For several sRNAs and their mRNA targets, translational inhibition in the absence of mRNA degradation is sufficient for the negative regulation of gene expression, and cleavage of the mRNA by RNase E is a subsequent step. For example, pairing of the SgrS sRNA with *ptsG* mRNA is sufficient to inhibit translation even if degradation of the *ptsG* mRNA is blocked (in an *rne* mutant). However, in a wild-type strain, pairing subsequently leads to degradation of the mRNA (51, 52). In a similar fashion, RyhB pairing with the *sodB* mRNA leads to inhibition of the translation of SodB whether or not the mRNA is degraded (52).

Is translational inhibition by an sRNA and the resulting absence of ribosomes on the mRNA sufficient to lead to degradation of the mRNA by RNase E? Normally, transcription and translation are tightly coupled in *E. coli*, and thus, most mRNAs may be covered with ribosomes soon after they emerge from RNA polymerase (Fig. 2*A*) (53, 54). There is a variety of evidence suggesting that blocking translation generally increases mRNA degradation, presumably because RNase E is able to bind to sites in the mRNA that would otherwise be protected by translating ribosomes (Fig. 2*B*) (reviewed in Ref. 2). However, there is also evidence that inhibition of translation is not always sufficient, suggesting that pairing has a second important role in recruiting RNase E. For *ptsG* mRNA, translational inhibition of the *ptsG* mRNA by addition of the translational inhibitor kasugamycin does not lead to destabilization of this mRNA unless SgrS is expressed (52). This suggests that pairing of SgrS with the *ptsG* mRNA leads to active recruitment of RNase E for degradation. Similarly, for RyhB regulation of *sodB*, a specific RNase E site far from the site of pairing was found to be necessary for *sodB* degradation, and blocking translation by inserting a stop codon early in the ORF was not sufficient to recruit RNase E; pairing by RyhB significantly improved RNase E-dependent cleavage (55).

Another consequence of sRNA-dependent decoupling of translation from transcription is exemplified by the regulation of the *chiPQ* mRNA by ChiX. The sRNA ChiX pairs with a region of the *chiPQ* mRNA that encompasses the RBS of *chiP* (56). Pairing of ChiX with the *chiPQ* mRNA leads to termination of the transcription of the mRNA by decoupling transcription and translation and therefore allowing entry of Rho onto the mRNA (Fig. 2*B*) (57). As a result, the downstream gene is not transcribed. This mechanism of negative regulation by decoupling of transcription and translation may be particularly important for polycistronic mRNAs.

Negative regulation of gene expression by an sRNA does not always result from translational inhibition. In *S. enterica* serovar Typhimurium, the MicC sRNA binds far downstream of the
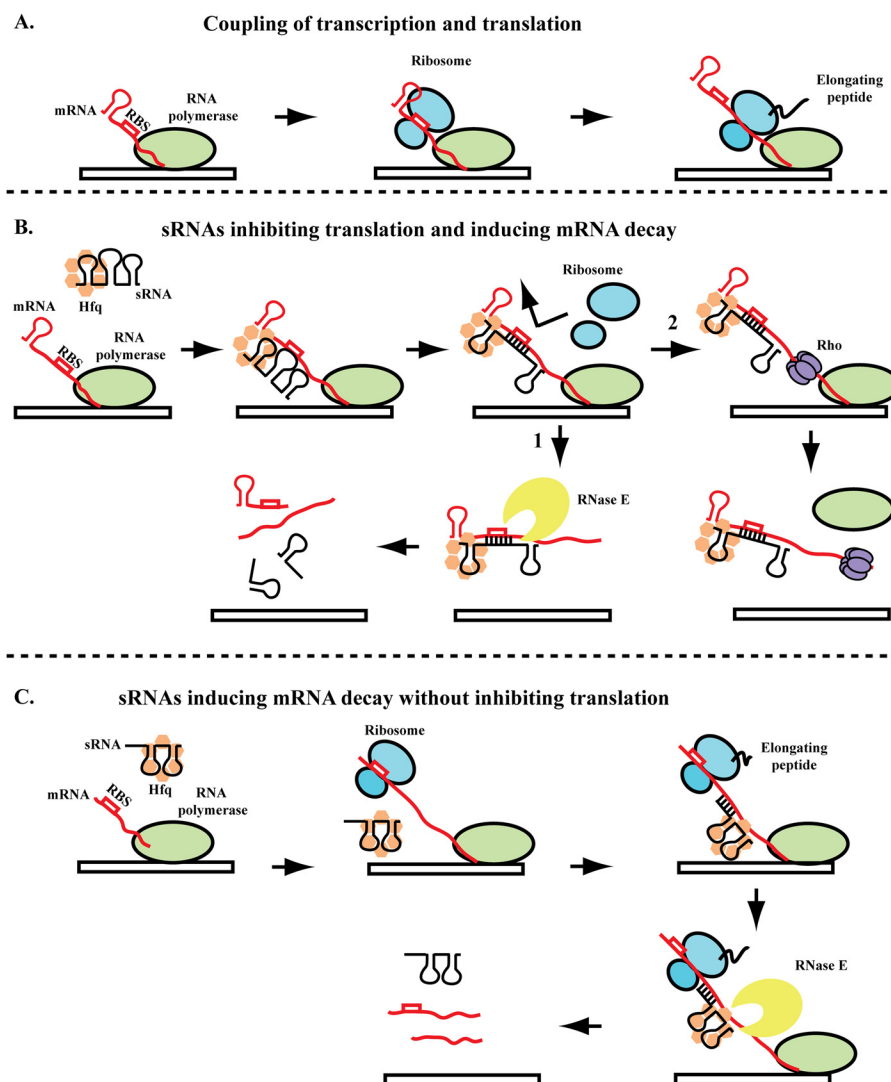
FIGURE 2. **Mechanisms of sRNA-mediated regulation.** *A*, the coupling of transcription by RNA polymerase (*green*) and translation by the ribosome (*blue*) in the absence of sRNA regulation. Growing evidence suggests the possibility of interactions between RNA polymerase and the ribosome to ensure coupling (see text). *B*, sRNAs can block ribosome entry by pairing with sequences at or near the RBS of the mRNA. Pairing of the sRNA with the target mRNA is facilitated by Hfq (*orange*). As a result of blocking ribosome binding, the sRNA can decouple transcription and translation. *Arrow 1*, the unprotected mRNA may then be subject to cleavage by RNase E (*yellow*). *Arrow 2*, in addition to access to RNase E, the untranslated RNA can also provide a binding site for Rho (*purple*), leading to transcription termination. *C*, alternatively, sRNAs may induce cleavage of the mRNA by recruiting RNase E, causing mRNA cleavage without inhibiting translation. Recruitment of RNase E may be through interactions with Hfq, the RNAs, or both.

start codon of *ompD*, which encodes an outer membrane protein. Binding of the sRNA does not disrupt translation of the *ompD* mRNA by ribosomes but leads to mRNA degradation (Fig. 2*C*) (41). The mechanism by which MicC stimulates degradation of the *ompD* mRNA has been recently elucidated. The cleavage of mRNAs by RNase E is stimulated by a monophosphate at the 5′-end of the sRNA. Bandyra *et al.* (58) have shown that, after pairing with the *ompD* mRNA, the 5′-monophosphate on MicC stimulates cleavage of the *ompD* mRNA. Stimulated cleavage requires pairing between the sRNA and mRNA. This type of regulation presumably allows RNase E to recognize target mRNAs that would otherwise lack an ideal RNase E-binding site. Regulation of *lpxR* by the MicF sRNA is also partially dependent on stimulation of RNase E cleavage, in this case, by changes in the folding of the *lpxR* mRNA upon MicF binding (59).

Interaction of RNase E with Hfq May Be Important for RNase E Recruitment

The regulatory examples discussed above all imply recruitment of RNase E as a result of sRNA-mRNA pairing. Thus, it might not be surprising if RNase E and Hfq interact directly. In fact, several co-immunoprecipitation studies have found that Hfq interacts with RNase E and other components of the degradosome, although whether this interaction is direct or via RNA (or both) remains unclear (33, 35, 60–62).

By co-immunoprecipitation experiments with crude extracts or purified Hfq and RNase E, Hfq was found to bind to a peptide composed of residues 711–844 of RNase E, and this interaction still occurred after treatment with RNases (35). These results suggest that the interaction between Hfq and RNase E is direct and not indirect through RNA. Consistent with an important site in this region, deletion of residues 701–845 of RNase E

caused a defect in regulation of the *ptsG* mRNA by SgrS (35). This deletion removes the region of RNase E containing the RhlB-binding site and an arginine-rich domain (AR2) involved in RNA binding but leaves the other arginine-rich binding domain (ARRBD) intact. Consistent with the idea that RhlB and Hfq occupy similar sites on RNase E, independent of RNA binding, overexpression of RhlB resulted in a decrease in the amount of Hfq bound to RNase E, and Hfq was pulled down with an RNase E derivative containing only the first 750 amino acids, lacking the AR2 domain but containing the ARRBD (35). These experiments do not rule out the possibility that either the ARRBD or AR2 domain is sufficient to bind Hfq through RNA.

Ikeda *et al.* (35) also noted that Hfq is likely to interact with RNase E at multiple sites, even if the site they identified is critical for regulation. These additional interactions may help to explain contrasting results obtained by Worrall *et al.* (62), who demonstrated *in vitro* that purified Hfq bound to a C-terminal fragment of RNase E from residues 628 to 843 but that this interaction did not occur unless RNA was present. This region does contain the two arginine-rich RNA-binding domains (Fig. 1*B*), and it is possible that the indirect binding of RNase E to Hfq by RNA can occur at lower Hfq concentrations than the direct binding reported by Ikeda *et al.* (35). It is certainly possible that both protein-protein interactions and RNA-mediated interactions take place *in vivo*. Future experiments should clarify this situation, but the data all point to an association of Hfq and RNase E that plays a role in negative regulation.

PNPase May Block RNase E Recruitment of Hfq-bound sRNAs Prior to mRNA Pairing

As discussed above, Hfq interacts directly or indirectly with the C-terminal scaffold domain of RNase E. In addition, degradation of the sRNA and target mRNA after pairing has been shown to require the C-terminal scaffold domain (35, 40–42). Together, these results suggest that Hfq may deliver the sRNA and target mRNA to the degradosome after pairing. If this is the case, what prevents Hfq from delivering the sRNA to the degradosome before the sRNA pairs with the mRNA?

For some sRNAs, PNPase blocks recruitment of Hfq-bound sRNAs by RNase E (42, 63). PNPase is a 3′–5′-exoribonuclease that catalyzes the decay of RNAs via a nucleophilic attack of the phosphodiester backbone with an inorganic phosphate. PNPase can also catalyze the reverse reaction, adding ribonucleotides to RNA, under conditions of high nucleotide diphosphate and low phosphate concentrations. PNPase can associate with the C-terminal scaffold domain of RNase E and with Hfq (32).

Two recent studies have shown that PNPase can protect sRNAs from premature degradation by RNase E (42, 63). In both studies, deletion of *pnp* resulted in a decreased stability of several sRNAs. Deletion of *pnp* also resulted in the abrogation of sRNA-dependent regulation, presumably because the sRNAs were unable to accumulate (42). In the absence of PNPase, these sRNAs were degraded by RNase E prior to pairing with target mRNAs. Premature degradation of sRNAs in the absence of PNPase required recruitment via the C-terminal domain of RNase E because deletion of this domain increased the stability of sRNAs in a *pnp* mutant (42). Presumably, it is Hfq-bound

sRNAs that are recruited to the C-terminal scaffold domain of RNase E in the absence of *pnp*.

It is not yet clear whether PNPase protects only a subset of Hfq-dependent sRNAs from premature degradation or protects them only under some conditions. PNPase also contributes to degradation of sRNAs, as expected for an exonuclease, although these data reflect studies done in stationary phase, whereas the studies in which PNPase protected sRNAs were done during exponential growth. Viegas *et al.* (64) demonstrated that PNPase is important for degradation of two sRNAs, the Hfq-dependent MicA sRNA and the Hfq-independent SraL sRNA in *S. enterica* serovar Typhimurium, in stationary phase. It was also found that PNPase is critical for the degradation of MicA and RybB sRNAs, again during stationary phase, because deletion of *pnp* increased their stability in *E. coli* (65). It appears that PNPase degrades sRNAs that are not bound to Hfq because the effect of a *pnp* deletion in stabilizing MicA sRNA was much greater in an *hfq* mutant (63). RyhB and SgrS accumulated to higher levels, and their stability was modestly higher in an *hfq pnp* double mutant compared with the stability in an *hfq* or a *pnp* single mutant, particularly in stationary phase (63). This result is different from that found in exponential phase in our studies (42). A possible explanation for the increased degradation of these sRNAs in the hfq^- pnp^+ strain is that PNPase acts as a polymerase, extending the 3′-tail of the sRNA and thus providing a foothold for 3′–5′-exoribonucleases, including PNPase itself; this polymerase activity is significantly increased in the absence of Hfq (32). PNPase may also act by regulating access of the sRNA to RNase E, known to degrade sRNAs in the absence of Hfq (39), protecting them when they are bound to Hfq and accelerating their recognition and/or degradation by RNase E in the absence of Hfq.

This was not the first evidence supporting a role of *pnp* in protecting RNAs from degradation. In a genome-wide expression profiling experiment, deletion of *pnp* was shown to result in a significant increase in the expression of >500 mRNAs, as might be expected if PNPase was important for degradation of these RNAs (66); some of these increases may also reflect failure of sRNA-based negative regulation. However, deletion of *pnp* also led to a substantial decrease in the expression of 140 genes, suggesting the possibility that PNPase also protects many mRNAs from degradation, directly or indirectly (via failure of sRNA positive regulation, for instance) (66). Our studies of mRNA targets of sRNA regulation also support a decrease in the steady-state level of some mRNAs in *pnp* mutants (42). Consistent with this idea of PNPase being able to protect RNAs from degradation, Briani *et al.* (67) showed that overexpression of the ribosomal protein S1 results in stabilization of mRNAs and that this stabilization requires PNPase. This is intriguing because S1 binds RNA with a similar sequence specificity as Hfq and clearly carries out RNA chaperoning activities independent of the ribosome (reviewed in Ref. 68). One could imagine that PNPase regulates the accessibility to RNase E of RNAs bound to both of these proteins. Although many of the details of how PNPase affects sRNA trafficking remain unexplored, these studies all suggest that Hfq, PNPase, and RNase E interact with each other in ways that have significant effects on the fate of

both sRNAs and their mRNA targets and that reflect previously unsuspected roles for PNPase (42, 66, 67).

Conclusions, Comparisons, and Future Directions

sRNA-dependent regulation is now well established in bacterial cells. The list of Hfq-dependent sRNAs in *E. coli* and *S. enterica* serovar Typhimurium is large, and each sRNA has multiple mRNA targets. Although these sRNA-mRNA pairs share dependence on some common machinery (primarily Hfq) for regulation to occur, it is becoming increasingly clear that there are many variations on how regulation actually occurs. These variations are helping us to understand more about how translational regulation in general can occur.

One important insight is the impact of the usual coupling of transcription and translation and how uncoupling this affects the ability of sRNAs to act (Fig. 2). The uncoupling of transcription and translation that underlies the ability of both RNase E and Rho to access the mRNA makes clear the important role that competition between sRNAs and ribosomes plays in translational regulation, and thus, ribosomes and their ability to load on a given mRNA must be considered as important components of the sRNA-dependent regulatory network. The growing understanding of ribosome occupancy on different mRNAs (see, for instance, Ref. 69) will help in unraveling these issues. Other protein participants in sRNA trafficking seem likely to be discovered in the near future. The complex interactions of the translation apparatus, the mRNA degradation apparatus, and the sRNAs themselves are likely to take longer to unravel.

To what extent do findings for bacterial sRNAs mirror our understanding of the eukaryotic microRNAs (miRNAs) and siRNAs (reviewed in Ref. 70)? In their mature form, miRNAs function as 21–23-nucleotide RNAs that can bind their target through imperfect complementarity, usually in the 3′-UTR, leading to gene silencing. For silencing to take effect, the mature miRNA must be presented to its targets in the context of a set of proteins called the RNA-induced silencing complex (RISC). The first job of RISC is to take the pre-miRNA exported from the nucleus and process it to a mature single-stranded miRNA. This task presumably is not essential for bacterial sRNAs that are frequently, if not always, active in their unprocessed form.

At the core of RISC are a glycine-tryptophan repeat-containing protein of 182 kDa (GW182) and the Argonaute protein (Ago), which includes domains for nucleic acid binding and a PIWI domain that has ribonuclease activity. RISC loaded with an miRNA can then bind to an mRNA target through RNA-RNA interactions. Presumably, RISC is important to stabilize and present the very short miRNA, a role that Hfq also plays with its longer sRNA substrates. RISC is also directly involved in the outcome of pairing/recruitment. If perfect complementarity exists between the miRNA and mRNA target, then annealing can induce endonuclease activity by Ago, and subsequent degradation of the target can occur. In the case of imperfect complementarity to a target mRNA, miRNA-mediated translational repression and/or subsequent deadenylation and decapping will occur, leading to mRNA decay (reviewed in Ref. 26). Ago proteins both present the sRNA to the mRNA and serve as the platform to recruit factors for RNA processing. Hfq

and RNase E may serve in similar capacities, with Hfq certainly promoting pairing. We understand less about how Hfq presents the paired RNAs to RNase E and how the RNase E scaffold acts to recruit other players.

As found with bacterial sRNA-mediated repression, multiple pathways of miRNA-mediated translational repression have been characterized, blocking initiation in multiple ways or, in some cases, blocking translation beyond initiation (reviewed in Ref. 70). For eukaryotic mRNAs, there is certainly no direct coupling of transcription and translation, but the coupling of maturation of mRNA and translation is achieved by the direct communication of the poly(A) tail with the initiation of translation.

It seems clear that prokaryotes and eukaryotes have evolved different machineries to process and present regulatory RNAs, but these sRNAs facilitate similar outcomes, well regulated control of translation, using machinery that is sensitive to the state of the mRNA and its ability to be translated and that can be tuned in multiple ways to fit the physiological requirements. Therefore, future lessons learned in eukaryotes may also hold true for prokaryotes and vice versa: what is true for an elephant may actually be true for *E. coli*.

REFERENCES

1. Jacob, F., and Monod, J. (1961) Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3,** 318–356
2. Gottesman, S. (2011) Roles of mRNA stability, translational regulation, and small RNAs in stress response regulation. in *Bacterial Stress Responses* (Storz, G., and Hengge, R. eds) 2nd Ed., pp. 59–73, ASM Press, Washington, D.C.
3. Fröhlich, K. S., and Vogel, J. (2009) Activation of gene expression by small RNA. *Curr. Opin. Microbiol.* **12,** 674–682
4. Waters, L. S., and Storz, G. (2009) Regulatory RNAs in bacteria. *Cell* **136,** 615–628
5. Carmichael, G. G., Weber, K., Niveleau, A., and Wahba, A. J. (1975) The host factor required for RNA phage Qβ RNA replication *in vitro*. Intracellular location, quantitation, and purification by polyadenylate-cellulose chromatography. *J. Biol. Chem.* **250,** 3607–3612
6. Wilusz, C. J., and Wilusz, J. (2005) Eukaryotic Lsm proteins: lessons from bacteria. *Nat. Struct. Mol. Biol.* **12,** 1031–1036
7. Vogel, J., and Luisi, B. F. (2011) Hfq and its constellation of RNA. *Nat. Rev. Microbiol.* **9,** 578–589
8. Brennan, R. G., and Link, T. M. (2007) Hfq structure, function and ligand binding. *Curr. Opin. Microbiol.* **10,** 125–133
9. Sun, X., Zhulin, I., and Wartell, R. M. (2002) Predicted structure and phyletic distribution of the RNA-binding protein Hfq. *Nucleic Acids Res.* **30,** 3662–3671
10. Schumacher, M. A., Pearson, R. F., Møller, T., Valentin-Hansen, P., and Brennan, R. G. (2002) Structures of the pleiotropic translational regulator Hfq and an Hfq-RNA complex: a bacterial Sm-like protein. *EMBO J.* **21,** 3546–3556
11. Link, T. M., Valentin-Hansen, P., and Brennan, R. G. (2009) Structure of *Escherichia coli* Hfq bound to polyriboadenylate RNA. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 19292–19297
12. Mikulecky, P. J., Kaw, M. K., Brescia, C. C., Takach, J. C., Sledjeski, D. D., and Feig, A. L. (2004) *Escherichia coli* Hfq has distinct interaction surfaces for DsrA, *rpoS* and poly(A) RNAs. *Nat. Struct. Mol. Biol.* **11,** 1206–1214
13. Otaka, H., Ishikawa, H., Morita, T., and Aiba, H. (2011) PolyU tail of rho-independent terminator of bacterial small RNAs is essential for Hfq

action. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 13059–13064

14. Sauer, E., and Weichenrieder, O. (2011) Structural basis for RNA 3′-end recognition by Hfq. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 13065–13070

15. Ishikawa, H., Otaka, H., Maki, K., Morita, T., and Aiba, H. (2012) The functional Hfq-binding module of bacterial sRNAs consists of a double or single hairpin preceded by a U-rich sequence and followed by a 3′ poly(U) tail. *RNA* **18,** 1062–1074

16. Zhang, A., Wassarman, K. M., Ortega, J., Steven, A. C., and Storz, G. (2002) The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. *Mol. Cell* **9,** 11–22

17. Brescia, C. C., Mikulecky, P. J., Feig, A. L., and Sledjeski, D. D. (2003) Identification of the Hfq-binding site on DsrA RNA: Hfq binds without altering DsrA secondary structure. *RNA* **9,** 33–43

18. Geissmann, T. A., and Touati, D. (2004) Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator. *EMBO J.* **23,** 396–405

19. Balbontín, R., Fiorini, F., Figueroa-Bossi, N., Casadesús, J., and Bossi, L. (2010) Recognition of heptameric seed sequences underlies multi-target regulation by RybB small RNA in *Salmonella enterica. Mol. Microbiol.* **78,** 380–394

20. Zhang, A., Schu, D. J., Tjaden, B. C., Storz, G., and Gottesman, S. (2013) Mutations in interaction surfaces differentially impact *E. coli* Hfq association with small RNAs and their mRNA targets. *J. Mol. Biol.*, doi: 10.1016/j.jmb.2013.01.006

21. Sauer, E., Schmidt, S., and Weichenrieder, O. (2012) Small RNA binding to the lateral surface of Hfq hexamers and structural rearrangements upon mRNA target recognition. *Proc. Natl. Acad. Sci. U.S.A.* **109,** 9396–9401

22. Soper, T. J., and Woodson, S. A. (2008) The *rpoS* mRNA leader recruits Hfq to facilitate annealing with DsrA sRNA. *RNA* **14,** 1907–1917

23. Salim, N. N., and Feig, A. L. (2010) An upstream Hfq binding site in the *fhlA* mRNA leader region facilitates the OxyS-*fhlA* interaction. *PLOS ONE* **5,** e13028

24. Beisel, C. L., Updegrove, T. B., Janson, B. J., and Storz, G. (2012) Multiple factors dictate target selection by Hfq-binding small RNAs. *EMBO J.* **31,** 1961–1974

25. Salim, N. N., Faner, M. A., Philip, J. A., and Feig, A. L. (2012) Requirements of upstream Hfq-binding (ARN)$_x$ elements in *glmS* and the Hfq C-terminal region for GlmS upregulation by sRNAs GlmZ and GlmY. *Nucleic Acids Res.* **40,** 8021–8032

26. Höck, J., and Meister, G. (2008) The Argonaute protein family. *Genome Biol.* **9,** 210.211–210.218

27. Wang, W., Wang, L., Zou, Y., Zhang, J., Gong, Q., Wu, J., and Shi, Y. (2011) Cooperation of *Escherichia coli* Hfq hexamers in DsrA binding. *Genes Dev.* **25,** 2106–2117

28. Moon, K., and Gottesman, S. (2011) Competition among Hfq-binding small RNAs in *Escherichia coli. Mol. Microbiol.* **82,** 1545–1562

29. Olejniczak, M. (2011) Despite similar binding to the Hfq protein, regulatory RNAs widely differ in their competition performance. *Biochemistry* **50,** 4427–4440

30. Hussein, R., and Lim, H. N. (2011) Disruption of small RNA signaling caused by competition for Hfq. *Proc. Natl. Acad. Sci. U.S.A.* **108,** 1110–1115

31. Updegrove, T. B., Correia, J. J., Chen, Y., Terry, C., and Wartell, R. M. (2011) The stoichiometry of the *Escherichia coli* Hfq protein bound to RNA. *RNA* **17,** 489–500

32. Mohanty, B. K., Maples, V. F., and Kushner, S. R. (2004) The Sm-like protein Hfq regulates polyadenylation-dependent mRNA decay in *Escherichia coli. Mol. Microbiol.* **54,** 905–920

33. Morita, T., Maki, K., and Aiba, H. (2005) RNase E-based ribonucleoprotein complexes: mechanical basis of mRNA destabilization mediated by bacterial noncoding RNAs. *Genes Dev.* **19,** 2176–2186

34. Windbichler, N., von Pelchrzim, F., Mayer, O., Csaszar, E., and Schroeder, R. (2008) Isolation of small RNA-binding proteins from *E. coli*: evidence for frequent interaction of RNAs with RNA polymerase. *RNA Biol.* **5,** 30–40

35. Ikeda, Y., Yagi, M., Morita, T., and Aiba, H. (2011) Hfq binding at RhlB-recognition region of RNase E is crucial for the rapid degradation of mRNAs mediated by sRNAs in *Escherichia coli. Mol. Microbiol.* **79,** 419–432

36. Carpousis, A. J., Luisi, B. F., and McDowall, K. J. (2009) Endonucleolytic initiation of mRNA decay in *Escherichia coli. Prog. Mol. Biol. Transl. Sci.* **85,** 91–135

37. Kushner, S. R. (2007) Messenger RNA decay. in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (Bock, A., Curtiss, R., III, Kaper, J. B., Karp, P. D., Neidhardt, F. C., Nyström, T., Slauch, J. M., Squires, C. L., and Ussery, D., eds) Chapter 4.6.4, ASM Press, Washington, D.C.

38. Carpousis, A. J. (2007) The RNA degradosome of *Escherichia coli*: a multiprotein mRNA-degrading machine assembled on RNase E. *Annu. Rev. Microbiol.* **61,** 71–87

39. Massé, E., Escorcia, F. E., and Gottesman, S. (2003) Coupled degradation of a small regulatory RNA and its mRNA targets in *Escherichia coli. Genes Dev.* **17,** 2374–2383

40. Desnoyers, G., and Massé, E. (2012) Noncanonical repression of translation initiation through small RNA recruitment of the RNA chaperone Hfq. *Genes Dev.* **26,** 726–739

41. Pfeiffer, V., Papenfort, K., Lucchini, S., Hinton, J. C. D., and Vogel, J. (2009) Coding sequence targeting by MicC RNA reveals bacterial mRNA silencing downstream of translational initiation. *Nat. Struct. Mol. Biol.* **16,** 840–846

42. De Lay, N., and Gottesman, S. (2011) Role of polynucleotide phosphorylase in sRNA function in *Escherichia coli. RNA* **17,** 1172–1189

43. Zhang, A., Wassarman, K. M., Rosenow, C., Tjaden, B. C., Storz, G., and Gottesman, S. (2003) Global analysis of small RNA and mRNA targets of Hfq. *Mol. Microbiol.* **50,** 1111–1124

44. Sittka, A., Sharma, C. M., Rolle, K., and Vogel, J. (2009) Deep sequencing of *Salmonella* RNA associated with heterologous Hfq proteins *in vivo* reveals small RNAs as a major target class and identifies RNA processing phenotypes. *RNA Biol.* **6,** 266–275

45. Chao, Y., Papenfort, K., Reinhardt, R., Sharma, C. M., and Vogel, J. (2012) An atlas of Hfq-bound transcripts reveals 3′-UTRs as a genomic reservoir of regulatory small RNAs. *EMBO J.* **31,** 4005–4019

46. Massé, E., Vanderpool, C. K., and Gottesman, S. (2005) Effect of RyhB small RNA on global iron use in *Escherichia coli. J. Bacteriol.* **187,** 6962–6971

47. Papenfort, K., Pfeiffer, V., Mika, F., Lucchini, S., Hinton, J. C. D., and Vogel, J. (2006) σ^E-dependent small RNAs of *Salmonella* respond to membrane stress by accelerating global *omp* mRNA decay. *Mol. Microbiol.* **62,** 1674–1688

48. Mandin, P., and Gottesman, S. (2009) A genetic approach for finding small RNA regulators of genes of interest identifies RybC as regulating the DpiA/DpiB two-component system. *Mol. Microbiol.* **72,** 551–565

49. Urban, J. H., and Vogel, J. (2009) A green fluorescent protein (GFP)-based plasmid system to study post-transcription control of gene expression *in vivo. Methods Mol. Biol.* **540,** 301–319

50. Bouvier, M., Sharma, C. M., Mika, F., Nierhaus, K. H., and Vogel, J. (2008) Small RNA binding to 5′ mRNA coding region inhibits translational initiation. *Mol. Cell* **32,** 827–837

51. Maki, K., Uno, K., Morita, T., and Aiba, H. (2008) RNA, but not protein partners, is directly responsible for translational silencing by a bacterial Hfq-binding small RNA. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 10332–10337

52. Morita, T., Mochizuki, Y., and Aiba, H. (2006) Translational repression is sufficient for gene silencing by bacterial small noncoding RNAs in the absence of mRNA destruction. *Proc. Natl. Acad. Sci. U.S.A.* **103,** 4858–4863

53. Burmann, B. M., Schweimer, K., Luo, X., Wahl, M. C., Stitt, B. L., Gottesman, M. E., and Rösch, P. (2010) A NusE:NusG complex links transcription and translation. *Science* **328,** 501–504

54. Proshkin, S., Rahmouni, A. R., Mironov, A., and Nudler, E. (2010) Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science* **328,** 504–508

55. Prévost, K., Desnoyers, G., Jacques, J.-F., Lavoie, F., and Massé, E. (2011) Small RNA-induced mRNA degradation achieved through both translation block and activated cleavage. *Genes Dev.* **25,** 385–396

56. Figueroa-Bossi, N., Valentini, M., Malleret, L., and Bossi, L. (2009) Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target. *Genes Dev.* **23,** 2004–2015

57. Bossi, L., Schwartz, A., Guillemardet, B., Boudvillain, M., and Figueroa-Bossi, N. (2012) A role for Rho-dependent polarity in gene regulation by a noncoding small RNA. *Genes Dev.* **26,** 1864–1873

58. Bandyra, K. J., Said, N., Pfeiffer, V., Górna, M. W., Vogel, J., and Luisi, B. F. (2012) The seed region of a small RNA drives the controlled destruction of the target mRNA by the endoribonuclease RNase E. *Mol. Cell* **47,** 943–953

59. Corcoran, C. P., Podkaminski, D., Papenfort, K., Urban, J. H., Hinton, J. C. D., and Vogel, J. (2012) Superfolder GFP reporters validate diverse new mRNA targets of the classic porin regulator, MicF RNA. *Mol. Microbiol.* **84,** 428–445

60. Morita, T., Kawamoto, H., Mizota, T., Inada, T., and Aiba, H. (2004) Enolase in RNA degradosome plays a crucial role in the rapid decay of glucose transporter mRNA in response to metabolic stress in *Escherichia coli. Mol. Microbiol.* **54,** 1063–1075

61. Butland, G., Peregrín-Alvarez, J. M., Li, J., Yang, W., Yang, X., Canadien, V., Starostine, A., Richards, D., Beattie, B., Krogan, N., Davey, M., Parkinson, J., Greenblatt, J., and Emili, A. (2005) Interaction network containing conserved and essential protein complexes in *Escherichia coli. Nature* **433,** 531–537

62. Worrall, J. A. R., Górna, M., Crump, N. T., Phillips, L. G., Tuck, A. C., Price, A. J., Bavro, V. N., and Luisi, B. F. (2008) Reconstitution and analysis of the multienzyme *Escherichia coli* RNA degradosome. *J. Mol. Biol.* **382,** 870–883

63. Andrade, J. M., Pobre, V., Matos, A. M., and Arraiano, C. M. (2012) The crucial role of PNPase in the degradation of small RNAs that are not associated with Hfq. *RNA* **18,** 844–855

64. Viegas, S. C., Pfeiffer, V., Sittka, A., Silva, I. J., Vogel, J., and Arraiano, C. M. (2007) Characterization of the role of ribonucleases in *Salmonella* small RNA decay. *Nucleic Acids Res.* **35,** 7651–7664

65. Andrade, J. M., and Arraiano, C. M. (2008) PNPase is a key player in the regulation of small RNAs that control the expression of outer membrane proteins. *RNA* **14,** 543–551

66. Mohanty, B. K., and Kushner, S. R. (2003) Genomic analysis of *Escherichia coli* demonstrates differential roles for polynucleotide phosphorylase and RNase II in mRNA abundance and decay. *Mol. Microbiol.* **50,** 645–658

67. Briani, F., Curti, S., Rossi, F., Carzaniga, T., Mauri, P., and Dehò, G. (2008) Polynucleotide phosphorylase hinders mRNA degradation upon ribosomal protein S1 overexpression in *Escherichia coli. RNA* **14,** 2417–2429

68. Hajnsdorf, E., and Boni, I. V. (2012) Multiple activities of RNA-binding proteins S1 and Hfq. *Biochimie* **94,** 1544–1553

69. Li, G.-W., Oh, E., and Weissman, J. S. (2012) The anti-Shine-Dalgarno sequence drives translational pausing and codon choice in bacteria. *Nature* **484,** 538–541

70. Fabian, M. R., and Sonenberg, N. (2012) The mechanics of miRNA-mediated gene silencing: a look under the hood of miRISC. *Nat. Struct. Mol. Biol.* **19,** 586–593