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Review

Contributions of the Hfq protein to translation regulation by small noncoding RNAs binding to the mRNA coding sequence

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The bacterial Sm-like protein Hfq affects the regulation of translation by small noncoding RNAs (sRNAs). In this way, Hfq participates in the cell adaptation to environmental stress, regulation of cellular metabolism, and bacterial virulence. The majority of known sRNAs bind complementary sequences in the 5'-untranslated mRNA regions. However, recent studies have shown that sRNAs can also target the mRNA coding sequence, even far downstream of the AUG start codon. In this review, we discuss how Hfq contributes to the translation regulation by those sRNAs which bind to the mRNA coding sequence.

Key words: Hfq, sRNA, mRNA, coding sequence, RNase E, ribosome

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INTRODUCTION

The Sm-like protein Hfq is a global regulator, which mediates the interactions between *trans*-encoded small noncoding RNAs (sRNAs) and their mRNA targets (Waters & Storz, 2009; Updegrove *et al.,* 2016). Hfq is conserved in more than half of known bacterial species, but its role in the interactions with small RNAs has been most thoroughly explored in the *Escherichia coli* and *Salmonella typhimurium* enterobacteria (Vogel & Luisi, 2011). sRNAs exert their control of gene expression by binding to partly complementary sequences in the target mRNAs (Melamed *et al.,* 2016), which can lead to changes in the access of the ribosome to the mRNA or can affect the mRNA stability (Waters & Storz, 2009). sRNAs participate in regulation of the cell's adaptation to changes in the environment, including regulation of the protein composition of the cell membrane (Chao & Vogel, 2016; Parker & Gottesman, 2016), metabolism of nitrogen compounds (Hao *et al.,* 2016), sugar metabolism (Beisel & Storz, 2011), regulation of extrachromosomal DNA elements (Cech *et al.,* 2014; Papenfort *et al.,* 2015), control of transcription and RNA decay (Lalaouna *et al.,* 2015; Fontaine *et al.,* 2016; Lee & Gottesman, 2016), biofilm formation (Jorgensen *et al.,* 2013), and the interactions with the host organism during infection (Chao & Vogel, 2010; Papenfort & Vogel, 2014; Kakoschke *et al.,* 2016).

The Hfq protein has a shape of a homohexameric ring with three distinct RNA binding sites on its surface, which allows it to simultaneously interact with several RNA molecules (Schumacher *et al.,* 2002; Mikulecky *et al.,* 2004; Link *et al.,* 2009; Sauer *et al.,* 2012). The site located on the distal face of the Hfq ring preferentially binds adenosine-rich sequences or the repeated trinucleotide ARN motifs (adenosine, purine, any nucleotide) that are mainly found in mRNAs (de Haseth & Uhlenbeck, 1980; Mikulecky *et al.,* 2004; Soper & Woodson, 2008; Salim *et al.,* 2012; Ellis *et al.,* 2015; Wroblewska & Olejniczak, 2016). The site on the proximal face is recognized by uridine-rich sequences, such as the 3'-terminal sRNA tails arising from the Rho-independent terminators of transcription (Otaka *et al.,* 2011; Sauer & Weichenrieder, 2011; Morita *et al.,* 2015). The third RNA binding site is located on the rim of the Hfq ring (Sauer *et al.,* 2012). The positively charged amino acids on the rim are essential for the influence of Hfq on RNA annealing (Panja *et al.,* 2013; Zheng *et al.,* 2016), while the adjacent negatively charged residues on the proximal face contribute to the specificity of Hfq binding to different RNA targets (Panja *et al.,* 2015). Although RNA molecules bind to Hfq with tight, sub-nanomolar affinities, they are rapidly recycled in competition with other RNA targets of Hfq (Fender *et al.,* 2010; Olejniczak, 2011; Malecka *et al.,* 2015; Santiago-Frangos *et al.,* 2016). The multiple RNA binding sites of Hfq allow it to use different binding modes in interactions with complementary sRNA and mRNA molecules to facilitate their pairing (Zhang *et al.,* 2013; Schu *et al.,* 2015).

Many of the Hfq-dependent small RNAs affect translation initiation by pairing in the area of the ribosome binding site in the 5'-untranslated regions of the mRNA molecules. Hfq contributes to this regulation in different ways. For example, the role of Hfq in the positive regulation of the *rpoS* mRNA translation is to rearrange the structure of this mRNA to facilitate binding of the DsrA sRNA to the 5'-UTR (Soper & Woodson, 2008; Soper *et al.,* 2010). The distortion of *rpoS* mRNA structure is induced by Hfq binding to an $(ARN)_4$ sequence, and the following binding of DsrA sRNA allows to shift the equilibrium between the ribosome-accessible and inaccessible conformations of this 5'-UTR leading to activation of the *rpoS* mRNA translation (Lease & Woodson, 2004; Soper & Woodson, 2008; Soper *et al.,* 2011; Peng *et al.,* 2014a; Peng *et al.,* 2014b). In the negative regulation of translation by sRNAs binding to the 5'-UTR of mRNA, Hfq also often contributes to sRNA annealing to mRNA (Moller *et al.,* 2002a; Zhang *et al.,* 2002; Geissmann & Touati, 2004). However, other modes of Hfq action are also possible. The Hfq protein is recruited by Spot42

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Abbreviations: ARN, a trinucleotide sequence consisting of adenosine, purine, any nucleotide; AU-rich sequence, adenosine and uridine-rich sequence; Hfq, Host factor for phage Q beta replication; 5'-UTR, 5'-untranslated region; RIL-seq, RNA interaction by ligation and sequencing; sRNA, small noncoding RNA

Table 1. sRNAs binding in the five-codon window of their target mRNA molecules.

sRNA	mRNA (bacterial species)	Hfq binds to sRNA/ mRNA	sRNA binding site in the CDS	Hfq's role	References
Spot42	*xylF* (*E. coli*)	+/+	+2–+40	stabilizes Spot42	(Beisel & Storz, 2011; Melamed *et al.,* 2016)
RybB	*ompW* (*S.* Typhimurium)	+/ n.d.	+3–+20	stabilizes RybB	(Papenfort *et al.,* 2006; Papenfort *et al.,* 2010)
DsrA	*hns* (*E. coli*)	+/+	+3–+19	stabilizes DsrA, translation inhibition is Hfq-dependent	(Sledjeski *et al.,* 2001; Lalaouna *et al.,* 2015; Melamed *et al.,* 2016)
RybB	*ompN* (*S.* Typhimurium)	+/+	+4–+11	stabilizes RybB, binds both RNAs using different sites	(Papenfort *et al.,* 2006; Bouvier *et al.,* 2008; Sittka *et al.,* 2008; Sauer *et al.,* 2012)
RybB	*ompS* (*S.* Typhimurium)	+/ n.d.	+7–+20	stabilizes RybB	(Papenfort *et al.,* 2006; Papenfort *et al.,* 2010)
RybB	*ompD* (*S.* Typhimurium)	+/+	+10–+26	stabilizes RybB, accelerates RybB annealing to mRNA	(Papenfort *et al.,* 2006; Papenfort *et al.,* 2010; Wroblewska & Olejniczak, 2016)
ArcZ	*tpx* (*S.* Typhimurium)	+/ n.d.	+10–+26	stabilizes ArcZ, essential for translation repression	(Sittka *et al.,* 2008; Papenfort *et al.,* 2009; Sittka *et al.,* 2009)
RybB	*chip* (*S.* Typhimurium)	+/ n.d.	+12–+18	stabilizes RybB	(Papenfort *et al.,* 2006; Balbontin *et al.,* 2010)
Spot42	*sthA* (*E. coli*)	+/+	+15–+22	stabilizes Spot42	(Moller *et al.,* 2002b; Beisel & Storz, 2011)
InvR	*ompD* (*S.* Typhimurium)	+/+	+15–+65	stabilizes InvR	(Pfeiffer *et al.,* 2007)
MicL	*lpp* (*E. coli*)	+/+	+16–+28	stabilizes MicL	(Guo *et al.,* 2014; Melamed *et al.,* 2016)

n.d. – no data

sRNA to the *sdhC* mRNA ribosome binding site to inhibit protein synthesis (Desnoyers & Masse, 2012). Hfq can also directly repress translation of other mRNAs by binding to their 5'-untranslated regions and interfering with translation initiation (Salvail *et al.,* 2013; Sonnleitner & Blasi 2014; Ellis *et al.,* 2015).

The Hfq protein also plays a role in protecting bound sRNA molecules from degradation before they pair with their mRNA targets (Saramago *et al.,* 2014). RNase E is a major enzyme responsible for RNA degradation and processing in *E. coli* and displays specificity towards single-stranded AU-rich sequences and 5'-monophosphorylated substrates (Saramago *et al.,* 2014; Frohlich *et al.,* 2016; Richards & Belasco, 2016). However, other RNases are also involved in regulation exerted by sRNAs (Andrade *et al.,* 2012). Beyond sRNA protection, Hfq can also contribute to sRNA-dependent mRNA decay by recruiting RNase E to mRNA (Ikeda *et al.,* 2011).

The mRNA coding sequence can also serve as a target for regulatory small RNAs (Bouvier *et al.,* 2008; Pfeiffer *et al.,* 2009; Gutierrez *et al.,* 2013; Papenfort *et al.,* 2013; Guo *et al.,* 2014; Bobrovskyy & Vanderpool, 2016; Melamed *et al.,* 2016). This is counterintuitive, because the elongating ribosome has a strong helicase activity and would be expected to unfold sRNA-mRNA complexes on its path (Takyar *et al.,* 2005; Qu *et al.,* 2011). However, a recent study using RIL-seq methodology revealed that the mRNA coding sequence is an important target of Hfq-bound sRNAs in the *E. coli* cells (Melamed *et al.,* 2016). These data are supported by two other Hfq profiling studies, which had shown that more than a third of the identified Hfq binding sites were located in the mRNA coding sequences (Tree *et al.,* 2014; Holmqvist *et al.,* 2016). The mechanisms used by several of those sRNAs have already been investigated, and they are discussed here to elucidate the possible contributions of Hfq to the regulation of translation by small RNAs binding to the mRNA coding sequences.

REGULATION IN THE FIVE-CODON WINDOW

The region of mRNA covered by the initiating ribosome consists of about 30 nucleotides, with mRNA entering the ribosome at about 15 nucleotides from the start codon (Huttenhofer & Noller, 1994; Yusupova *et al.,* 2001). The sRNAs which target the 5'-untranslated region often interfere with the ribosome access to the Shine-Dalgarno sequence or the start codon. However, pairing of sRNAs to the region of the mRNA coding sequence protected by the initiation complex can also lead to translation repression (Table 1). The first described example of such regulation was the *Salmonella ompN* mRNA, whose translation is controlled by RybB sRNA (Bouvier *et al.,* 2008). The results of toeprinting experiments and *in vitro* translation assays had shown that RybB sRNA, which binds at +4 to +11 of the *ompN* coding sequence, interferes with translation at the stage of initiation. Further experiments using a complementary oligonucleotide indicated that the first 15 nucleotides of the coding sequence constitute a region in which sRNA binding leads to translation repression. This region has been called a five-codon window for the mRNA translation regulation by sRNAs (Bouvier *et al.,* 2008). Further studies also identified other mRNAs which are targeted in this region by RybB (Balbontin *et al.,* 2010; Papenfort *et al.,* 2010), ArcZ (Papenfort *et al.,* 2009), Spot42 (Beisel & Storz, 2011), and DsrA sRNAs (Lalaouna *et al.,* 2015).

Table 2. sRNAs that bind within the first 100 nt of the mRNA coding sequence.

sRNA	mRNA (bacterial species)	Hfq binds to sRNA/ mRNA	sRNA binding site	Hfq's role	References
RybB	*ompA* (*S.* Typhimurium)	+/+	+21–+32	stabilizes RybB	(Vytvytska *et al.,* 2000; Papenfort *et al.,* 2006; Papenfort *et al.,* 2010)
SgrS	*manX* (*E. coli*)	+/+	+24–+37	stabilizes SgrS	(Morita *et al.,* 2005; Rice & Vanderpool, 2011)
MgrR	*soxS* (*E. coli*)	+/+	+29–+44	stabilizes MgrR, necessary for MgrR-dependent *soxS* repression	(Moon and Gottesman 2009; Lee and Gottesman 2016; Melamed *et al.,* 2016)
SdsR	*ompD* (*S.* Typhimurium)	+/+	+39–+51	accelerates SdsR annealing	(Frohlich *et al.,* 2012; Wroblewska & Olejniczak, 2016)
RybB	*fadL* (*S.* Typhimurium)	+/+	+43–+50	stabilizes RybB, accelerates RybB annealing	(Papenfort *et al.,* 2006; Papenfort *et al.,* 2010; Groszewska *et al.,* 2016)
SgrS	*purR* (*E. coli*)	+/+	+49–+67	stabilizes SgrS, SgrS recruits Hfq to translation initiation region	(Morita *et al.,* 2005; Bobrovskyy & Vanderpool, 2016)
MicC	*ompD* (*S.* Typhimurium)	+/+	+67–+78	stabilizes MicC, accelerates MicC annealing	(Pfeiffer *et al.,* 2009; Wroblewska & Olejniczak, 2016)
Spot42	*icd* (*E. coli*)	+/+	+75–+86	stabilizes Spot42	(Moller *et al.,* 2002b; Wright *et al.,* 2013; Melamed *et al.,* 2016)
Spot42	*gdhA* (*E. coli*)	+/+	+80–+94	stabilizes Spot42	(Moller *et al.,* 2002b; Wright *et al.,* 2013; Melamed *et al.,* 2016)
OxyS	*fhlA* (*E. coli*)	+/+	–9––15 +20–+28	affects OxyS stability, accelerates OxyS annealing	(Argaman & Altuvia, 2000; Zhang *et al.,* 2002; Salim & Feig, 2010; Henderson *et al.,* 2013)
MicF	*lpxR* (*S.* Typhimurium)	+/ n.d.	–11––3 +70–+104	stabilizes MicF, facilitates MicF annealing	(Urban & Vogel, 2007; Corcoran *et al.,* 2012)

n.d. – no data

The fact that MicL represses translation by binding at +16 to +28 of the *lpp* coding sequence (Guo *et al.,* 2014) suggests that sRNAs that bind in the region immediately adjacent to the five-codon window could also interfere with the translation initiation, presumably through a steric effect of the remaining sRNA structure.

Besides repression of translation initiation, the sRNA binding in the five-codon window can also lead to mRNA decay. Experiments with untranslated mutants of the *lpp* mRNA indicated that the role of MicL sRNA was to repress *lpp* mRNA translation, and that the observed mRNA decay was the result of the interrupted translation (Guo *et al.,* 2014). Similarly, binding of the DsrA sRNA to the sequence immediately downstream of the AUG start codon of *hns* mRNA induced translation repression, which was shown by using *in vitro* translation assays (Lalaouna *et al.,* 2015). In this case, binding of DsrA was followed by the cleavage of *hns* mRNA at +131 of the coding sequence, which was dependent on RNase E and the degradosome (Lalaouna *et al.,* 2015). These data suggest that the primary effect of sRNA binding in the five-codon window and the adjacent area is the repression of translation initiation, while the following mRNA decay could result from the lack of mRNA protection by the ribosome when translation is stopped.

Hfq facilitates pairing of the RybB sRNA to the *ompD* mRNA (Wroblewska & Olejniczak, 2016). Repression of this mRNA's translation is induced by RybB binding at +10 to +26 of its coding sequence (Bouvier *et al.,* 2008). Experiments using purified Hfq and RNAs had shown that Hfq bound both RNAs and increased the rate of their annealing (Wroblewska & Olejniczak, 2016). A study using short complementary fragments of RybB and *ompD* mRNA suggested that Hfq rearranged the structures of both interacting RNAs, which facilitated their pairing. Moreover, Hfq mutagenesis experiments indicated that Hfq was binding RybB by its proximal face and the opposite distal face was used to interact with *ompD* mRNA (Wroblewska & Olejniczak, 2016). This mode of Hfq binding to the interacting RNAs is consistent with the model proposed by previous studies on the role of Hfq in RybB interactions with *ompN* mRNA (Sauer & Weichenrieder, 2011; Sauer *et al.,* 2012). Overall, these data suggest that Hfq facilitates annealing of RybB to the five-codon window of the regulated mRNAs by binding to both interacting RNAs and rearranging their structures.

Besides its role in annealing of sRNAs to the five-codon window, Hfq also contributes to the stability of sRNAs and the decay of their mRNA targets. For example, Hfq protects MicL sRNA from degradation, which enables this sRNA to repress the *lpp* translation (Guo *et al.,* 2014). Hfq is also necessary for regulation exerted by several other sRNAs targeting the five-codon window, such as Spot42 binding to *xylF* and *sthA* mRNAs (Beisel & Storz, 2011), and ArcZ binding to *tpx* (Papenfort *et al.,* 2009). Although in these cases a precise role of Hfq in the exerted regulation has not been investigated. it seems likely that it could also involve protecting the sRNAs from degradation by cellular ribonucleases. Moreover, Hfq can also participate in the degradosome recruitment to mRNA (Ikeda *et al.,* 2011), which ensures the irreversibility of the sRNA mediated translation repression. Consistently, Hfq was necessary for repression of the *hns* mRNA translation which is partly dependent on the cleavage of this mRNA by RNase E (Lalaouna *et al.,* 2015).

REGULATION BY sRNAs BINDING DEEPLY IN THE CODING SEQUENCE

Beyond the footprint of the initiating ribosome, functional sRNA binding sites were identified in the re-

Table 3. sRNAs that bind deeply in the mRNA coding sequence.

sRNA	mRNA (bacterial species)	Hfq binds to sRNA/ mRNA	sRNA binding site	Hfq's role	References
SR1	*ahrC* (*B. subtilis*)	+/+	multiple predicted sites at +80 to +325	Hfq affects *ahrC* mRNA translation *in vivo*	(Heidrich *et al.,* 2006; Heidrich *et al.,* 2007)
DsrA	*rbsD* (*E. coli*)	+/+	+298–+313	stabilizes DsrA, facilitates DsrA annealing	(Sledjeski *et al.,* 2001; Lalaouna *et al.,* 2015; Melamed *et al.,* 2016)
SdsR	*mutS* (*E. coli*)	+/+	+1385–+1409	necessary for SdsR-dependent repression of MutS translation	(Tsui *et al.,* 1997; Gutierrez *et al.,* 2013; Melamed *et al.,* 2016)
SgrS	*yigL* (*S.* Typhimurium)	+/ n.d.	+935–+955 of *pldB*	stabilizes SgrS, activation of *yigL* is dependent on Hfq	(Morita *et al.,* 2005; Papenfort *et al.,* 2013)

n.d. - data

gions ranging from just outside of the five-codon window region to as far as 1400 nt in the coding sequence (Frohlich *et al.,* 2012; Gutierrez *et al.,* 2013) (Table 2, 3). The majority of these sRNAs bind within the first 100 nucleotides of the coding sequence. This group includes SdsR and MicC sRNAs binding to the *ompD* mRNA (Pfeiffer *et al.,* 2007; Pfeiffer *et al.,* 2009; Frohlich *et al.,* 2012), RybB sRNA binding to *ompA* and *fadL* mRNAs (Papenfort *et al.,* 2010), MicF binding to *lpxR* mRNA (Corcoran *et al.,* 2012; Holmqvist *et al.,* 2012), MgrR binding to *soxS* mRNA (Lee & Gottesman, 2016), SgrS binding to *manX* mRNA (Rice & Vanderpool, 2011) and to *purR* mRNA (Bobrovskyy & Vanderpool, 2016), and others (Table 2). However, a few sRNAs bind even further downstream in the coding sequence (Table 3). Among them, DsrA sRNA recognizes the *rbsD* mRNA at +298 to +313 (Lalaouna *et al.,* 2015), and SdsR binds to the *mutS* mRNA at +1385 to +1409 (Gutierrez *et al.,* 2013).

Even sRNAs binding outside of the footprint of the initiation ribosome can affect the initiation step of translation. Repression of the *manX* mRNA translation by SgrS binding at +24 to +37 of its coding sequence was not dependent on the mRNA degradation, which suggested that the primary effect of the sRNA binding was translation inhibition (Rice & Vanderpool, 2011). In a different example, binding of the SR1 sRNA to the coding sequence of *ahrC* mRNA from *Bacillus subtilis* resulted in translation repression, which was mediated by the mRNA structure rearrangements (Heidrich *et al.,* 2007). Another mechanism was proposed for control of the *purR* mRNA translation by the SgrS sRNA. Binding of this sRNA beyond 40 nt in the coding sequence resulted in the recruitment of Hfq to the translation initiation region, where it directly interfered with translation (Bobrovskyy & Vanderpool 2016). Additionally, secondary sRNA binding sites in the coding sequence could enhance the effect of primary binding sites in the ribosome binding region, as it was observed in the regulation of *fhlA* and *lpxR* mRNAs by OxyS and MicF sRNAs, respectively (Argaman & Altuvia, 2000; Corcoran *et al.,* 2012). These data suggest that sRNAs that bind deeply in the coding sequence can indirectly affect the mRNA translation initiation.

Translation repression by sRNAs targeting the mRNA coding sequence could also result from sRNA-induced mRNA degradation, as it was proposed for the MicC dependent regulation of the *ompD* mRNA (Pfeiffer *et al.,* 2009; Wagner, 2009). RNase E is a major bacterial enzyme involved in RNA decay. It can access its RNA substrates either via internal AU-rich binding sites or, more efficiently, through interaction with the 5'-terminal monophosphate groups (Richards & Belasco 2016). It has been proposed that the MicC sRNA, by binding to the *ompD* mRNA, recruits RNase E to induce rapid mRNA degradation (Pfeiffer *et al.,* 2009; Bandyra *et al.,* 2012). The mRNA decay dependent on RNase E has also been reported as a result of the SdsR binding to *ompD* (Frohlich *et al.,* 2012), MicF binding to *lpxR* (Corcoran *et al.,* 2012), and RybB binding to *ompA* and *fadL* mRNA (Papenfort *et al.,* 2010). However, sRNA binding can also activate translation by interfering with RNase E-dependent mRNA decay. For example, RNase E-dependent cleavage of the *pldB* mRNA enables the SgrS sRNA binding to the 3' end of *pldB* mRNA coding sequence, which in turn protects this bicistronic transcript from further decay, thus enabling continued translation of the downstream *yigL* cistron (Papenfort *et al.,* 2013). It is worth noting that RNase E is not the only ribonuclease involved in the decay of sRNA repressed transcripts (Saramago *et al.,* 2014). For example, although RNase E is required to release *rbsD* from polycistronic mRNA, it is not sufficient to degrade this mRNA, and it was proposed that an alternative RNase could be important for the *rbsD* decay (Lalaouna *et al.,* 2015).

A frequent theme in translation regulation by sRNAs that bind in the coding sequence is the important role of the mRNA structure rearrangements. For example, the *lpxR* mRNA structure rearrangement by the MicF sRNA affects its stability by exposing a region containing nucleotides +A82 and +U83 to the RNase E cleavage (Corcoran *et al.,* 2012). Similarly, the *rbsD* mRNA structure rearrangement upstream of the DsrA sRNA binding site promotes a rapid degradation of this transcript (Lalaouna *et al.,* 2015). In another example, rearrangement of the o*mpD* mRNA structure by the MicC sRNA results in the increased conformational flexibility of a region downstream of the sRNA binding site (Wroblewska & Olejniczak, 2016). Finally, rearrangement of the *ahrC* structure upon SR1 sRNA binding was implicated in the repression of translation initiation (Heidrich *et al.,* 2007).

Hfq binds MicC sRNA with sub-nanomolar affinity and accelerates its annealing to the *ompD* mRNA coding sequence (Wroblewska & Olejniczak, 2016). It was found that the role of Hfq was mainly to overcome the energetic barrier of the MicC sRNA structure, in agree-

ment with the fact that the MicC binding site in *ompD* was located in a partly unstructured region, likely accessible to sRNA pairing. It was also proposed that the long AU-rich sequence in the 5'-UTR of *ompD* mRNA served as the Hfq binding site essential for the MicC sRNA annealing to the coding sequence (Wroblewska & Olejniczak, 2016). Comparison of the Hfq-dependent annealing of the RybB and MicC sRNAs to the *ompD* mRNA, and the DsrA sRNA annealing to the *rpoS* mRNA, suggests that the Hfq contributions are individually tuned, depending on the structures of the interacting RNAs, to achieve their accelerated annealing (Soper & Woodson, 2008; Soper *et al.,* 2011; Wroblewska & Olejniczak, 2016).

The Hfq protein also contributes to the translation regulation by other sRNAs binding deeply in the coding sequence. Hfq stabilizes the MicF sRNA, facilitates this sRNA's binding to the *lpxR* mRNA, and is required for MicF-dependent regulation of *lpxR* expression (Urban & Vogel, 2007; Corcoran *et al.,* 2012). Lack of Hfq prevents DsrA dependent repression of *rbsD*, probably because of the lowered DsrA stability (Sledjeski *et al.,* 2001; Lalaouna *et al.,* 2015). In another example, the mismatch-repair activity of MutS is restored in Δ*hfq* mutant, which suggests the involvement of Hfq in SdsR-*mutS* interactions (Tsui *et al.,* 1997; Gutierrez *et al.,* 2013). Hfq also contributed to activation of the *yigL* mRNA expression (Papenfort *et al.,* 2013). Moreover, Hfq is directly responsible for efficient inhibition of *pur*R translation after recruitment by SgrS (Bobrovskyy & Vanderpool, 2016). As recent high-throughput studies discovered numerous binding sites of Hfq alone in the coding sequences, as well as new binding sites of Hfq-bound sRNAs, it is likely that further research will widely expand our knowledge on the mechanisms used by Hfq in the regulation of translation (Tree *et al.,* 2014; Holmqvist *et al.,* 2016; Melamed *et al.,* 2016).

CONCLUSIONS

Recent data has shown that the mRNA coding sequence is an important target for small regulatory RNAs in bacteria. The major mechanism of action of those sRNAs, which bind within the first five codons of an mRNA, is inhibition of the translation initiation step. When sRNAs bind outside of the footprint of the initiating ribosome, their action typically involves mRNA degradation, although there are also examples of the primary effect on translation inhibition. The data presented in this review indicate that Hfq can contribute to the regulation exerted by binding to the coding sequence in different ways. It protects the sRNAs from degradation, accelerates their annealing to mRNAs, and participates in recruiting RNases to the mRNAs that are repressed. Hfq may also induce rearrangements of the RNA structure, which is determined by individual structural features of the interacting RNAs. However, Hfq can also directly interfere with translation after it is recruited by a regulatory RNA. As the recent studies identified numerous new Hfq binding sites in the coding sequences of mRNAs, it is likely that new Hfq contributions to the complex interactions regulating bacterial metabolism will be revealed.

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