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On the facultative requirement of the bacterial RNA chaperone, Hfq

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The pleiotropic post-transcriptional regulator Hfq is an RNA chaperone that facilitates pairing interactions between small regulatory RNAs (sRNAs) and their mRNA targets in several bacteria. However, this classical pattern, derived from the *Escherichia coli* model, is not applicable to the whole bacterial kingdom. In this article we discuss the facultative requirement for Hfq for sRNA–mRNA duplex formation among bacteria and the specific features of the Hfq protein and RNA duplexes that might account for the dispensability or requirement of the chaperone. Apparent links between the need for Hfq, the GC content of bacterial genomes and the free energy of experimentally validated sRNA–mRNA pairing interactions are presented.

Is Hfq involved in all sRNA–mRNA interactions?

Bacterial small ribonucleic acids (sRNAs) that are not ribosomal, transfer or messenger RNAs play central roles in the regulation of gene expression in response to environmental changes [1]. Some sRNAs are also implicated in bacterial virulence [2]. The majority of the known sRNAs interact near, or at, the translation start site of their dedicated mRNA targets to exert mostly negative effects by RNA silencing, inducing mRNA decay and/or translation inhibition. Overlapping networks of interactions are common and experimentally validated pairings are usually found to be imperfect and to contain mismatches and unpaired nucleotides. In some bacterial species, such interrupted pairings are assisted by a dedicated RNA chaperone known as Hfq (host factor required for phage Q β RNA replication) that belongs to the Sm family of proteins [3–5].

Approximately half of the sequenced bacterial genomes encode a Hfq homolog [4]. Hfq proteins contain an N-terminal α -helical domain followed by an antiparallel five-stranded β -sheet and form stable hexameric ring-shaped structures of identical subunits [5]. They preferentially bind to A/U-rich single strands close to hairpins in RNAs [6]. Hfq is one of the most abundant proteins in *Escherichia coli* (30 000–60 000 per cell [7]) where it modulates the stability, translation and polyadenylation of many mRNAs [8], as well as RNA processing events. Defects including reduced growth, impaired resistance to various stresses and altered virulence are detected in *E. coli* cells lacking Hfq [9]. However, in some bacteria, such as *Staphylococcus aureus*, *hfq* is transcribed at very low

levels during growth [10], although sRNA–mRNA interactions are known to regulate the expression of several genes in this microorganism [11].

In this article we discuss the role of the bacterial Hfq protein in RNA-mediated gene regulation and highlight its dispensability in numerous cases. For our purpose, only experimentally supported sRNA–mRNA pairings (evidenced by structural probing and/or extended mutational analysis) are provided as examples. Structural variations in Hfq proteins throughout phylogeny are also considered. Here we propose that the need for Hfq is linked to the GC content of the bacterial genomes and the free energy of experimentally supported sRNA–mRNA pairing interactions.

Functions of the Hfq protein in sRNA-mediated gene regulation

E. coli is a paradigm for the importance of Hfq in sRNA-based gene regulation. At least 22 *E. coli* sRNAs require Hfq to function [12], including all *trans*-acting antisense sRNAs with the exceptions of the IstR-1/*tisB* [13], IpeX/*ompC* [14] and SymR/*symE* RNA hybrids [15] (by convention, hybrids are named in the form sRNA/*mRNA*). The sRNAs base pair with their target mRNAs and modulate gene expression by modifying mRNA decay and/or translation initiation. In *E. coli*, Hfq induces structural changes in the target mRNA and/or the sRNA. A study using real-time fluorescence resonance energy transfer (FRET) measurements on the interaction between truncated versions of the DsrA sRNA and the *rpoS* mRNA showed that Hfq accelerates strand exchange and subsequent annealing between the sRNA and its target mRNA [16]. The protein strongly enhances the rate of sRNA–mRNA duplex formation, enabling the sRNA to compete efficiently with ribosome binding.

Glossary

Antisense RNA: an RNA that interacts with a complementary mRNA target to modify its expression.

Degradosome: a multi-protein complex involved in the degradation of various RNAs.

Riboregulation: regulation exerted by an RNA.

RNA silencing: downregulation of gene expression by an RNA molecule.

RNome: all the nonconventional RNAs (mRNAs, tRNAs and rRNAs excluded) expressed by an organism.

Sm family of proteins: RNA-binding proteins that are involved in mRNA splicing in eukaryotes and archaea.

Trans-acting RNAs: RNAs that bind target mRNA sequences via imperfect pairings to regulate their expression levels.

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In *Salmonella enterica* serovar *Typhimurium*, high-throughput pyrosequencing technology has recently identified several sRNAs that are enriched by co-immunoprecipitation with Hfq [17]. In an independent study, Viegas and collaborators showed that a Hfq mutation reduces the half-life of two sRNAs expressed in this bacterium, SraL and MicA [18]. Interestingly, these sRNAs are conserved in several enterobacteria. The steady-state levels of two *Pseudomonas aeruginosa* sRNAs, PhrD and PhrS, are also both decreased by half in a *hfq* mutant, whereas their half-life remains unchanged [19]. This suggests that Hfq affects their transcription levels, although this might be an indirect effect. It should be noted that a lower level of a particular sRNA in a *hfq* mutant does not necessarily imply a role for Hfq in the formation of the corresponding sRNA–mRNA hybrid. In *E. coli*, however, most of the sRNAs requiring Hfq for their functions have a shorter half-life in *hfq* deletion strains [20]. In several gammaproteobacteria, Hfq is required for numerous sRNA-based gene regulations (Figure 1a) [1].

In *E. coli*, there is a functional link between Hfq and the single-strand specific endoribonuclease E (RNase E). Gene silencing by sRNAs is triggered by a ribonucleoprotein complex containing RNase E that interacts with Hfq to cause rapid target mRNA degradation and translation

repression [21]. Hfq interacts with the C-terminal domain of RNase E and the RNase cleaves the target mRNAs [21] to initiate their rapid decay. The Hfq binding sites on sRNAs coincide with the RNase E cleavage sites [22]. Therefore, Hfq might protect sRNAs from endonucleolytic attack, which would explain why Hfq increases the half-life of most sRNAs with which it interacts [20]. The protein also stabilizes Hfq-interacting sRNAs in bacteria other than *E. coli*, such as *Listeria monocytogenes* [23] and *P. aeruginosa* [24]. In cases for which Hfq is involved in sRNA–mRNA interactions, it can facilitate recruitment of RNase E from the degradosome for mRNA decay (exceptions exist, such as the *E. coli* sRNA Spot42, which does not induce degradation of its target mRNA *galK* [20]). In *E. coli*, degradation of repressed mRNAs is probably a consequence of ribosome exclusion rather than the primary event because translational repression can occur without mRNA destabilization [25], implying that RNase E might be dispensable for translation silencing. There is an interesting parallel with the situation in *S. aureus*: regulatory RNAIII (an sRNA) causes direct translation repression of target mRNAs and induces their degradation in a process dependent on the double-strand specific endoribonuclease III (RNase III) [11]. RNAIII-dependent inhibition of ribosome loading onto the target mRNAs is coupled to RNase

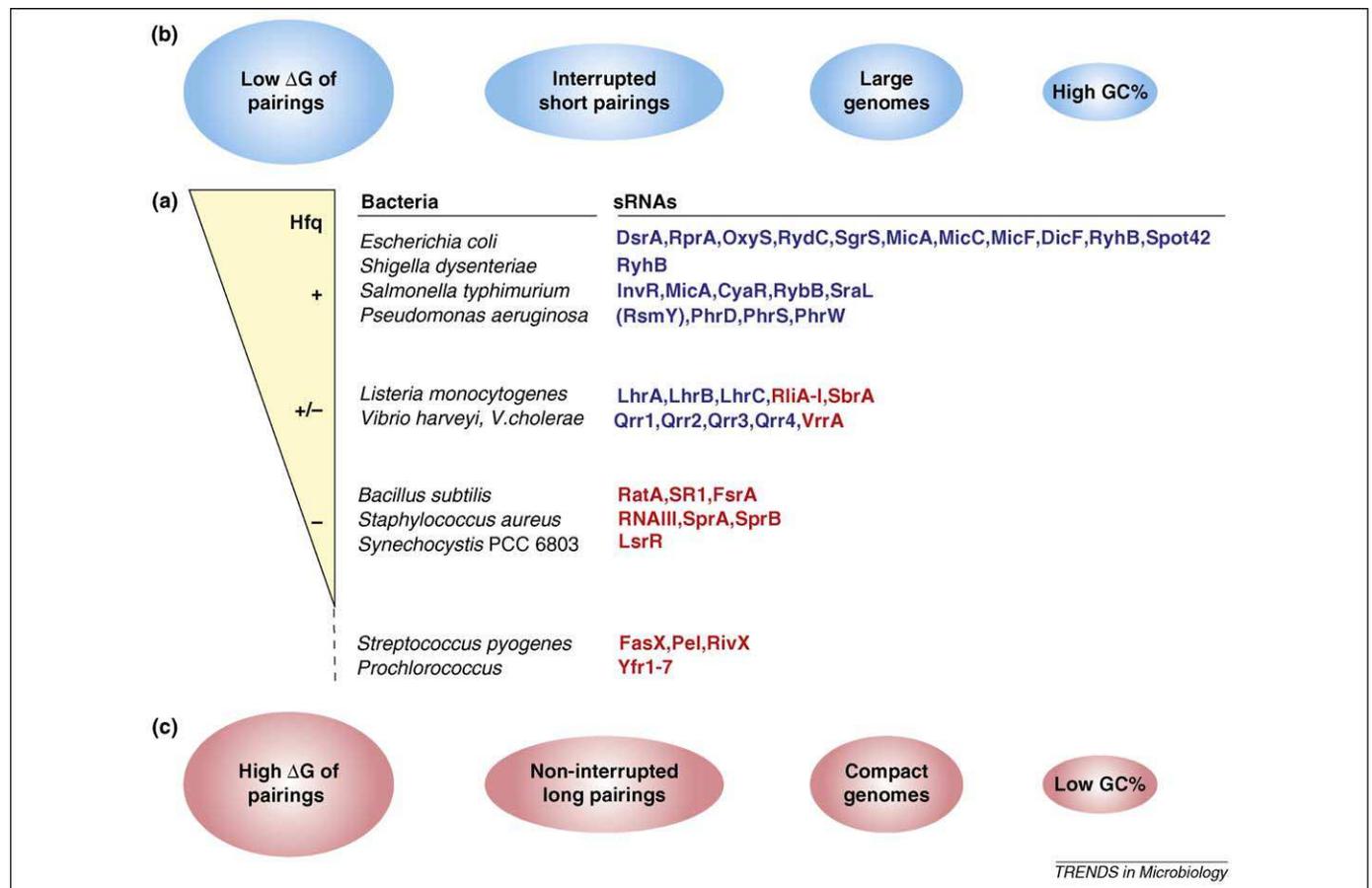


Figure 1. Variable requirement for Hfq in sRNA-mediated gene regulations in bacteria. (a) List of bacteria and sRNAs organized according to their Hfq requirement. With the exception of RsmY (shown in parentheses because it requires Hfq to function but traps regulatory proteins [55]), only sRNAs that regulate the expression of target mRNAs are considered. Hfq is required for the function of sRNAs colored in blue and dispensable for the function of those colored in red. The yellow triangle represents incremental involvement of Hfq. The *hfq* gene is missing in *S. pyogenes* and in most strains of *Prochlorococcus*. Note that in *E. coli* [56] and *S. typhimurium* [17] additional sRNAs interact with Hfq *in vivo*. (b,c) Parameters to consider for the facultative involvement of the Hfq protein in sRNA–mRNA interactions. The size of the circles reflects the relative importance of each parameter.

III-dependent cleavage, probably to render translation arrest irreversible.

In the same bacterium, Hfq can be required for some sRNAs and dispensable for others

In the human pathogen *Vibrio cholerae* and the marine bacterium *Vibrio harveyi*, Hfq creates a regulatory switch with four redundant sRNAs (Qrr1–Qrr4) impacting gene expression at high cellular density [26]. At least some of the Qrr RNAs in *V. cholerae* act by base pairing on multiple target mRNAs and require Hfq for function [27]. Another sRNA from *V. cholerae*, VrrA, downregulates *ompA* porin mRNA by base pairing with the 5' region of the mRNA [28]. However, VrrA-mediated regulation of OmpA expression occurs in the absence of Hfq *in vivo*, indicating that the protein is dispensable for the action of VrrA [28].

This non-uniform action of Hfq also seems to be true for *L. monocytogenes*. In this Gram-positive food-borne pathogen, three sRNAs (LhrA–LhrC) co-immunoprecipitate with Hfq [23], suggesting that the protein participates in their functions. However, RliB, RliE and RliI sRNAs from the same bacterium are able to interact with their mRNA targets *in vitro* without Hfq [29]. It should be noted, however, that a possible *in vivo* role for Hfq cannot be ruled out for these sRNAs based only on *in vitro* studies. The functions of these sRNAs are unclear and minor effects on expression levels of their putative target mRNAs were detected after sRNA overexpression, suggesting indirect regulation. Another recently identified sRNA, SbrA, which is part of the σ^B regulon (involved in response to stress) of *L. monocytogenes*, does not interact with Hfq and most probably functions independently of this RNA chaperone [30]. The above examples from *Vibrio* and *Listeria*, as well as the cases from *E. coli* (IstR, IpeX and SymE) already mentioned, illustrate how non-uniform the involvement of Hfq can be in sRNA–mRNA mediated regulations within the same bacterium.

Hfq as a dispensable factor in sRNA-controlled gene regulations

In the low-GC Gram-positive *S. aureus*, the *hfq* gene is present but expressed at a very low level during growth [10], which contrasts with the abundance of the Hfq protein in *E. coli*. However, *S. aureus* expresses numerous sRNAs [31,32] with at least one, RNAIII, forming antisense pairings with several mRNAs [11]. The involvement of Hfq in RNAIII-mediated gene regulation was addressed experimentally but remains unclear. According to co-immunoprecipitation and band shift experiments, RNAIII binds specifically to Hfq *in vivo* and *in vitro* [33]. However, Hfq does not facilitate complex formation between RNAIII and one of its target mRNAs (*spa*) and *hfq* deletion does not affect the *in vivo* level of *spa* mRNA or that of RNAIII [10]. Concerning additional mRNA targets of RNAIII, it has been shown that Hfq is not involved in translational repression of *rot* mRNA [34] or in duplex formation between *sa1000* mRNA and RNAIII [11]. This raises the question of the role of Hfq in the stability and function of other sRNAs in *S. aureus*.

Additional sRNAs are expressed by *S. aureus* [32], some of which (SprA and SprB) seem to regulate *in vivo* the expression of mRNAs by antisense pairing (Shabelskaya

and Felden, unpublished observations). As assayed by gel retardation, complexes between either SprA or SprB transcripts and purified *S. aureus* Hfq seem to form, but they might be non-specific and they can be destabilized by addition of an excess of non-relevant RNAs such as tRNAs (Jousselin *et al.*, unpublished observations). In addition, Northern blotting experiments revealed that Hfq does not seem to influence *in vivo* levels of SprA and SprB during both exponential and stationary growth phases (Jousselin *et al.*, unpublished observations). Moreover, SprA interacts *in vitro* with its predicted SA2216 mRNA target in the absence of Hfq [32]. Finally, after testing more than 1000 different growth conditions, no detectable phenotypic difference was observed between the *hfq* deletion mutant and wild type *S. aureus* [10]. All these data suggest that Hfq is dispensable for the known cases of sRNA-controlled gene regulation in *S. aureus*.

The chromosome of *Bacillus subtilis* encodes a Hfq-like RNA chaperone, YmaH [35], and several sRNAs under sporulation control [36]. SR1 is an sRNA involved in regulation of arginine catabolism by preventing translation of the *ahrC* mRNA, encoding a transcriptional activator. The YmaH protein is dispensable for complex formation between SR1 and *ahrC* mRNA *in vitro* and it does not influence the stability of SR1 *in vivo*, confirming its dispensability [37]. Structural probes revealed that the 3'-terminal stem-loop of SR1 unfolds to interact with *ahrC* mRNA [38]. FsrA, another sRNA expressed by *B. subtilis*, represses expression of mRNAs encoding iron-containing enzymes such as succinate dehydrogenase (*sdhC*) by pairing with *sdhC* mRNA [39]. Its function does not require a contribution from YmaH. Therefore, the Hfq homolog in *B. subtilis* is not involved in the *in vivo* stability of known sRNAs. An as yet uncharacterized factor is possibly required to facilitate the interaction *in vivo*.

A Hfq homolog was identified in *Synechocystis* sp. PCC 6803 and there is also an antisense RNA (IsrR) that negatively controls expression of the accessory photosynthesis protein IsiA [40]. Preliminary results argue against a role of the Hfq homolog in riboregulation of photosynthesis because IsiA and IsrR transcript levels are not altered in a Δhfq mutant. However, Hfq plays critical roles in the expression of motility *slr* genes in *Synechocystis* [41]. These specific examples indicate that Hfq, or Hfq homologs, can be dispensable for sRNA stability *in vivo*. Again, this contrasts with the situation in Gram-negative *E. coli*, in which Hfq influences the stability of most of the *trans*-acting sRNAs *in vivo*.

Rationalizing the facultative requirement for Hfq in bacterial sRNA–mRNA interactions

Genomic GC content

The implication of Hfq in sRNA–mRNA interactions varies among bacteria and seems to be somewhat connected to the overall GC content of the genomic DNA (Table 1). It is striking that most known *trans*-acting antisense sRNAs in *E. coli* (with a 50% overall GC content) need Hfq for function [12], as is the case for the other known sRNAs in gammaproteobacteria (50–67% overall GC content) [17,19]. However, for some bacteria with a GC content of ~50%, such as *Vibrio cholerae* (47%), the involvement of

Table 1. Relations between genome size, overall GC content and involvement of Hfq in RNA-controlled gene regulations in several bacteria

Bacterial species	Genome size (Mb)	GC (%)	Phylum	References
Hfq generally involved in sRNA function				
<i>Caulobacter crescentus</i>	4	67	Proteobacteria (α)	[57]
<i>Pseudomonas aeruginosa</i>	6	66	Proteobacteria (γ)	[19]
<i>Neisseria meningitidis</i>	2.2	51	Proteobacteria (β)	[58]
<i>Escherichia coli</i> ^a	4	50	Proteobacteria (γ)	[13–15]
<i>Salmonella typhimurium</i>	4.8	45	Proteobacteria (γ)	[17,59,60]
Hfq involved in the function of some sRNAs				
<i>Vibrio cholerae</i>	3	47	Proteobacteria (γ)	[26,28]
<i>Vibrio harveyi</i>	3.2	47	Proteobacteria (γ)	[26]
<i>hfq</i> gene present but seems to be dispensable				
<i>Bacillus subtilis</i>	4.2	43	Firmicutes	[38,39]
<i>Staphylococcus aureus</i>	2.8	32	Firmicutes	[11,33]
<i>Synechocystis</i> PCC6803	3	47.7	Cyanobacteria	[41]
<i>Prochlorococcus marinus</i>	1.6	31	Cyanobacteria	[49,50]
<i>hfq</i> gene absent				
<i>Streptomyces coelicolor</i> ^b	8	72	Actinobacteria	[61]
<i>Chlamydia trachomatis</i>	1	41	Chlamidiae	[62]
<i>Helicobacter pylori</i>	1.6	39	Proteobacteria (ϵ)	[1]
<i>Streptococcus pyogenes</i>	1.8	38	Firmicutes	[52–54]
<i>Enterococcus faecalis</i>	3.2	37	Firmicutes	[1]
<i>Borrelia burgdorferi</i>	0.9	26	Spirochaetes	[51]

^aIn *E. coli*, most sRNAs require Hfq for their functions, although three exceptions are known (see the text).

^bThe genome of *S. coelicolor*, with a high GC content, lacks *hfq* although it encodes several sRNAs; however, the functions of these sRNAs are unknown and might not depend on mRNA–sRNA pairing interactions.

Hfq is variable. Nevertheless, when considering bacteria with low-GC values, such as *S. aureus* (32% GC), Hfq seems to be dispensable. It seems that most bacteria requiring Hfq for some of their sRNA-controlled gene regulation possess an overall GC content $\geq 50\%$ (Table 1). A possible explanation for this observation is that a higher GC content in interacting sRNAs and mRNAs is expected to result in more stable conformations, which would need to be loosened by a chaperone for a productive interaction to occur. We therefore hypothesize that low-GC bacteria usually do not need Hfq to enhance sRNA–mRNA duplex formation for efficient gene regulation (Figure 1b,c).

Extended pairing might overcome the requirement for Hfq

In *E. coli*, Hfq-dependent sRNA–mRNA duplexes are formed between relatively short RNA sequences interrupted by mismatches [42,43]. For instance, the experimentally supported RyhB/*sodB* and OxyS/*fhlA* interactions only involve 9 and 7 bp, respectively (Table 2). In these cases, Hfq enhances the rate of duplex formation between the interacting RNAs.

By contrast, some sRNA–mRNA hybrids form in the absence of the Hfq protein *in vivo* and *in vitro*, as is the case for several mRNA targets of RNAIII in *S. aureus* [11]. These sRNA–mRNA duplexes are usually longer than those requiring Hfq in *E. coli*. It seems that long duplexes might have sufficient stability, with RNA chaperones being dispensable. As examples, the interactions (experimentally supported by structural probing) between *rot*, *spa* and *sa1000* mRNAs and RNAIII involve 33, 44 and 50 bp, respectively [11]. These extended pairings seem to overcome the requirement for Hfq, as is also the case for the long and uninterrupted sRNA–mRNA hybrids in *E. coli* plasmids and antisense sRNAs [44].

Table 2 provides examples of sRNA–mRNA interactions in various bacteria that are experimentally supported by either structural probing and/or mutagenesis. In each case, we calculated the free energy of the interaction using the *RNAup* software, which considers the energy necessary to open the binding site and the energy gained from hybridization [45]. Remarkably, we found that pairing interactions with the lowest ΔG values (RyhB/*sodB*, GcvB/*dppA*, OxyS/*fhlA*, GcvB/*oppA*) need the contribution of Hfq for their regulation, whereas the Hfq protein is dispensable for those with the highest ΔG values (RNAIII/*sa1000* and IstR-1/*tisAB*). Therefore, we propose that the role of Hfq in bacterial sRNA–mRNA interactions depends, at least in part, on the free energy for pairings (Figure 1b,c). However, the ΔG values calculated should be confirmed experimentally by melting temperature measurements for each sRNA–mRNA duplex.

Importance of the Hfq structure

Structural variations among Hfq proteins throughout bacterial phylogeny might also account for their different contributions in sRNA–mRNA interactions. Hfq proteins display a conserved common core but considerable variation at their C-terminal ends, with the gammaproteobacteria possessing the longest C-terminal extensions [4]. Interestingly, it has been shown that a C-terminal extension of *E. coli* Hfq constitutes an mRNA interaction surface because a C-terminal truncated Hfq variant binds sRNAs but not mRNAs [46]. The Hfq proteins from *S. aureus* and *B. subtilis* have very short C-termini, so it could be speculated that they are unable to interact efficiently with the mRNA targets of the sRNAs, which might contribute to their dispensability for sRNA–mRNA interactions in these organisms. Nevertheless, the smallest known Hfq, which occurs in the archeon *Methanocaldococcus jannaschii*,

Table 2. Relation between the free energy (ΔG) calculated for sRNA–mRNA pairings [63] and implication of Hfq in RNA-controlled gene regulations for RNA duplexes validated experimentally by structural probing and/or mutagenesis

sRNA–mRNA	Experimentally supported sRNA–mRNA pairings ^a	Pair length (bp)	GC (%)	ΔG (kcal/mol)	Refs
Hfq required					
RyhB/ <i>sodB</i> ^b	<i>sodB</i> 5'-AGUAGCAAUGUCAUU-3' RyhB 3'-CACUCGUUAACAGCAC-5'	9	44	-5.5	[42]
GcvB/ <i>dppA</i> ^b	GcvB 5' UACGGUUGUGAUG UGUGUUUGUUGUUUGCAAU 3' <i>dppA</i> 3' AGUAAGAAGACGAGGUUAACACUAC AUACAACAAAACGGGAAACG 5'	13	31	-8.96	[60,64]
OxyS/ <i>fhlA</i> ^b	OxyS 5' UUUUGCGGAUCUCCAGGAUCCGC 3' <i>fhlA</i> 3' GUAAAACAAGAAGGUCUUCUGUGGCGUUU 5'	7	57	-10.5	[65]
GcvB/ <i>oppA</i> ^b	GcvB 5' UACGGUUGUGAUGUUG UGUUGUUUGCAAUUGGUC 3' <i>oppA</i> 3' AAAAAGCACUACA AUACACAACAUGAGGUGA 5'	17	23	-11.2	[60,64]
PtsG/ <i>sgrS</i> ^b	<i>ptsG</i> 5'-GCACCCAUACUCAGGAGCACUCUCAUUUAUGUUUAG-3' <i>sgrS</i> 3'-UGUGGUUAUGAGUCAGUGUGU-ACUACGUCGCUUCA-5'	23	39	-12.2	[43]
Spot42/ <i>galK</i> ^b	Spot42 5' GUAGGGUACAGAGGUAGAUGUUCUAUCUUUCAGACC UUUUACUUCACGUAUUCGGAUUUGGCUGAA 3' <i>galK</i> 3' CGUCCAUCCGUUUA AACACAAAAGAAAGUCUGAGUAAAG AAUGUGA GGCCUAAGCG	40	35–50	-16	[66]
OmpA/ <i>micA</i> ^b	<i>ompA</i> 5'-GAUGAUACGAGGCGC-3' <i>MicA</i> 3'-CUACUAUUGUUUCGCG-5'	16	43	-20	[67]
Hfq not required					
FsrA/ <i>sdhC</i> ^c	<i>sdhC</i> 5'-ACUUAUCAACAGGGGU GGGAACAGAGUUUUUUUUUC-3' <i>fsrA</i> 3'-AGAAUAGACUAUCGCCCA C CCGUUGUCUCUCAUCGAAGAG-5'	42	20	-18	[39]
RNAIII/ <i>spa</i> ^d	<i>spa</i> 5'-ACAAUCAUACAGGGGG U--- AUUAAUUUGAAAAAGAAAACAUUUUAUCAAUUCGUAAA3' RNAIII 3'-AGUUAAGUAAAAACCCCUA UAAUUAAACUUUUUUUUAAA- UAAAUAGUUUA UAUUUUA-5'	44	15.9	-22.5	[33]
RNAIII/ <i>sa1000</i> ^{d,e}	SA1000 5'-UAUUUGAGGAUAGGUGUUUAUUUAUUUGAAAAAGAAUUUUAUUGGGAAAUCAAU-3' RNAIII 3'-AUAAAACCC-----CCUACAAUAAUUAUACUUUUUUU-AAAAUAAUAG UUAU---UUA-5'	50	10	-34.5	[11]
IstR-1/ <i>tisAB</i> ^b	IstR-1 5' GUUGACAUAUACAGUGUGCUU 3' <i>tisAB</i> 3' CAACUGUAUUGUGUCACACGAC 5'	21	39	-35	[13]

^aSR1/*ahrC* and *rot*/RNAIII were not included in the table because the free energy for these two pairing interactions could not be calculated. SR1/*ahrC* relies on several short experimentally supported interrupted nucleotide stretches, some located at long distances from one another [38]. In addition, the experimentally supported loop–loop interactions between *rot* mRNA and RNAIII occur between distant portions of each of the two RNA partners [11].

^bFrom *E. coli*.

^cFrom *B. subtilis*.

^dFrom *S. aureus*.

^e*sa1000* is a fibrinogen-binding mRNA.

binds efficiently to *E. coli* sRNAs and possesses the ability to promote riboregulation via intermolecular base pairing and can complement, at least in part, a number of phenotypes displayed by an *E. coli* *hfq* knockout strain [47].

Another important structural difference between *E. coli* and *S. aureus* Hfq proteins, which most likely impacts their functions, concerns their electrostatic potential energy surfaces and their proposed RNA binding sites. For *E. coli* Hfq, the positive electrostatic surface of the trough that connects the proximal and distal faces is in sharp contrast

to the same area on *S. aureus* Hfq, which shows a negative electrostatic surface [48]. Variability in the Hfq core barrel could also account for non-conservation of the RNA chaperone function.

Bypassing Hfq for sRNA functions

In bacteria expressing an RNome, keeping a functional Hfq chaperone is probably an advantage to enable a large set of sRNA–mRNA interactions to occur, including those that are energetically unfavorable. It is remarkable that some

bacteria, such as *S. aureus*, possess a copy of the *hfq* gene but the encoded protein does not seem to play any significant role in the cell; this is in agreement with the very low transcription levels, as discussed in a previous section. It is possible that some unidentified mutations in the promoter of the *hfq* gene might be responsible for the weak transcription yields. Alternatively, *hfq* could be expressed at high levels under restricted, and currently unknown, growth conditions.

The *hfq* gene could be in the process of being erased in certain bacterial lineages. In *Prochlorococcus*, *hfq* is present in only two out of 12 sequenced strains [49]. Nevertheless, the naturally *hfq*-deficient *Prochlorococcus* MED4 expresses at least 24 sRNAs [50]. The absence of *hfq* in MED4 suggests that either the antisense sRNAs detected function without chaperone support or that a novel non-Hfq chaperone is yet to be identified.

Despite the absence of *hfq* in its genome, the etiological agent of Lyme disease, *Borrelia burgdorferi*, contains several sRNAs [51]. Hfq homologs are also absent in several low-GC Gram-positive bacteria such as *Streptococcus pyogenes*, which expresses at least three sRNAs: FasX [52], RivX [53] and Pel [54]. All three sRNAs regulate the expression of virulence factors. The mechanism of action of these sRNAs is unknown and their functions might not require the help of a chaperone. Some bacteria lacking *hfq* (Table 1) have evolved towards minimal compact genomes and can bypass the need for the protein or any other chaperones in their riboregulation, probably because of evolutionary constraints linked to their ecological niches.

Concluding remarks and future directions

It is possible that additional chaperones could substitute for Hfq in bacteria lacking this protein. Database searches in *S. aureus* genomes for genes encoding Sm-like domains (which are structural signatures for RNA chaperones) reveal no other candidates than *hfq*. However, Gaballa and colleagues have recently reported that, in *B. subtilis*, an operon expresses three small basic proteins postulated to function as RNA chaperones (FbpA–FbpC) needed for the FsrA sRNA to regulate *sdhC* expression [39]. These proteins are candidate substitutes for Hfq; a further search for proteins other than Hfq able to facilitate sRNA–mRNA interactions could lead to interesting findings. The requirement or dispensability of protein chaperones for a specific sRNA might depend on the pairings involved with each target mRNA and on the conformations of the interacting RNAs.

Based on an extensive set of experimental data collected from various bacterial species, we propose that a link exists between the need for Hfq in sRNA–mRNA interactions and (i) the overall GC content of bacterial genomes, (ii) the free energy for sRNA–mRNA pairing interactions, (iii) genome size and (iv) structural variations among Hfq proteins. Although none of these parameters, individually, can be used to make detailed predictions on the involvement of Hfq for a specific case, we suggest that they should be considered in furthering our understanding of the variable implication of Hfq in sRNA–mRNA regulation.

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