Spectroscopic observation of RNA chaperone activities of Hfq in post-transcriptional regulation by a small non-coding RNA

Véronique Arluison1, Sungchul Hohng2, Rahul Roy3, Olivier Pellegrini1, Philippe Régnier1 and Taekjip Ha2, 3,*

1Institut de Biologie Physico-Chimique, CNRS UPR 9073 conventionnée avec l’Université Paris 7, 13 rue P. et M. Curie, 75005 Paris, France, 2Department of Physics and Howard Hughes Medical Institute and 3Center for Biophysics and Computational Biology, University of Illinois, Urbana-Champaign, Urbana, Illinois 61081, USA

Received November 15, 2006; Revised December 8, 2006; Accepted December 8, 2006

ABSTRACT

Hfq protein is vital for the function of many non-coding small (s)RNAs in bacteria but the mechanism by which Hfq facilitates the function of sRNA is still debated. We developed a fluorescence resonance energy transfer assay to probe how Hfq modulates the interaction between a sRNA, DsrA, and its regulatory target mRNA, rpoS. The relevant RNA fragments were labelled so that changes in intra- and intermolecular RNA structures can be monitored in real time. Our data show that Hfq promotes the strand exchange reaction in which the internal structure of rpoS is replaced by pairing with DsrA such that the Shine-Dalgarno sequence of the mRNA becomes exposed. Hfq appears to carry out strand exchange by inducing rapid association of DsrA and a premelted rpoS and by aiding in the slow disruption of the rpoS secondary structure. Unexpectedly, Hfq also disrupts a preformed complex between rpoS and DsrA. While it may not be a frequent event in vivo, this melting activity may have implications in the reversal of sRNA-based regulation. Overall, our data suggest that Hfq not only promotes strand exchange by binding rapidly to both DsrA and rpoS but also possesses RNA chaperoning properties that facilitates dynamic RNA–RNA interactions.

INTRODUCTION

RNA molecules serve as important regulatory factors in a variety of cellular processes. Examples include small interfering and micro RNA in eukaryotes and non-coding small RNA (sRNA) and riboswitches in prokaryotes. Protein–RNA interactions form the basis of RNA-based regulation of gene expression, but our understanding of its details at the molecular level is still poor. Fluorescence resonance energy transfer (FRET) is a powerful tool to study intermolecular interactions and intramolecular conformational changes, and therefore it serves as a desirable technique to investigate the regulatory mechanisms involving RNA–protein interactions. Here, we have developed FRET assays to probe how E. coli Hfq protein modulates the interaction between a sRNA and its target mRNA.

Hfq was originally discovered in E. coli as a host factor for Q-β-repliase (1), but analysis of hfq mutations suggested its involvement in many other metabolic pathways. Indeed, Hfq is now known to be a pleiotropic regulator that modulates the stability as well as translational activity of several mRNAs (2–4). The role of Hfq in translational regulation has been the focus of much attention lately since many sRNAs have been identified to require Hfq for their activity. For example, Hfq is involved in the post-transcriptional regulation of the rpoS gene (encoding the σ70 RNA polymerase subunit involved in bacterial stress response) (5). Hfq modulates rpoS expression by altering the binding of sRNAs such as DsrA, OxyS and RprA (6–8). Other examples of protein pathways regulated by Hfq-mediated coupling of a regulatory sRNA to a target mRNA include OxyS...fhlA, and Spot42...galK, where mRNA targets are italicized (reviewed in ref. (9)). Although it is likely that only a fraction of sRNA molecules that require Hfq as an additional factor have been identified, it is already clear that many of these sRNAs act by base-pairing with mRNA (10–12).
Hfq binds both sRNA and its corresponding mRNA simultaneously, forming a ternary complex and it was suggested that the enhanced local concentration of the RNA leads to final annealing of the two RNA which then exposes the mRNA coding sequence for translation (13,14). It has also been proposed that Hfq acts by changing the RNA structure (15,16). Furthermore, Hfq was shown to rescue a group I intron splicing intermediate trapped in a misfolded form in vivo (17) and hence qualifies as a RNA chaperone (18). It is likely that RNA chaperone activities of Hfq are also involved in translational regulation by sRNA (15,19).

Our current molecular understanding of Hfq activity largely stems from recent findings that it is a Sm-like protein. The most thoroughly characterized Sm proteins are those that form the cores of eukaryotic spliceosomal ribonucleoproteins implicated in pre-mRNA splicing. The Sm domain is highly conserved across many species and its wide phylogenetic distribution underlines its likely role in early evolution of RNA metabolism (20–22). In the case of Hfq, the relationship between eubacterial protein and Sm topology was first suggested by weak sequence conservation and subsequently confirmed by crystallographic structures of S. aureus, E. coli and P. aeruginosa Hfq proteins (23–26). Eukaryotic and archaeal Sm proteins form closed rings composed of seven monomers (identical or different) (27–29), whereas Hfq forms homo-hexameric rings. The toroidal-shaped Hfq ring is ~70Å in diameter (7,30), with a central cationic pore that forms the RNA-binding site. The structure of S. aureus Hfq-AU5G revealed RNA bound to one side of Hfq in a circular manner with each nucleotide stacking on the side chain of an aromatic residue (Tyr or Phe 42 for S. aureus and E. coli Hfq, respectively) (24,25). Furthermore, recent work has also provided compelling evidence that Hfq has multiple RNA binding sites (14,31).

In this study, we have focused on DsrA...Hfq...rpoS interactions to examine the chaperoning role of Hfq in promoting intermolecular base-pairing. It is generally accepted that, in the absence of stress conditions, the translation of rpoS mRNA is repressed by a stem region upstream of the AUG start codon that sequesters the Shine-Dalgarno sequence. The current model proposes that stress results in stimulation of base pairing between rpoS and DsrA, at least in part by increasing the synthesis of DsrA. DsrA...rpoS pairing allows disruption of the stem in rpoS and makes its ribosome binding site accessible (Figure 1a).

In these in vitro assays, however, it was not clear how the RNA chaperone activities of Hfq are related to the enhanced DsrA...rpoS interaction.

Here, we have developed real-time FRET assays to monitor the influence of Hfq on DsrA...rpoS annealing. Our studies show that Hfq not only binds rapidly to both DsrA and pre-melted rpoS sequence but also acts as a RNA chaperone which modulates the RNA secondary structures in a sequence-specific manner. A model is proposed for how Hfq helps in annealing DsrA and rpoS.

![Figure 1.](https://academic.oup.com/nar/article-abstract/35/3/999/2401405)
METHODS

Unless otherwise specified, all enzymes and chemicals were either from Sigma or Merck-Biochemicals.

RNA sequences

We used the following RNA sequences for FRET measurements (5′→3′): Cy5-DsrA, Cy5-AACACAU CAGAUUUCCUGUGUAACGAAUUUUUAAG; Cy3-rpoSI, biotin-AUUUUGAAAUUCGUAACAGG GGAAUCGGUAACCC-Cy3; Cy5.5-rpoSI, Cy5.5- CAAAGGCAUCGGUAGCGCCACCUAUAGA GUCAGAAU. Cy5-DsrA and Cy3-rpoSI were purchased from Dharmacon and deprotected according to the manufacturer’s recommendations. For Cy5.5-rpoSI, we labelled an amine-reactive RNA (IDTDNA) with NHS ester Cy5.5 following the scheme below.

Cy5.5 dye labelling

Here, 3.2 µl of RNA (4.2 mM in H2O), 5.6 µl of H2O, and 11.2 µl of amine-reactive Cy5.5 dissolved in DMSO (18 µg/µl) was added to 60 µl of sodium tetraborate buffer (0.1 M, pH 8.5), and incubated overnight at 4°C. Labelled RNA was recovered by ethanol precipitation.

RNA annealing

For RNA duplex melting experiments, two different RNA complexes were annealed. For rpoSI+II, we mixed 10 µl of Cy3-rpoSI (40 µM in T50 (10 mM Tris pH 8.0, 50 mM NaCl)) and 10 µl Cy5.5-rpoSI (100 µM in T50) and slowly cooled the mixture from 90°C to room temperature. After the sample reached room temperature (~4 h), we incubated it for another four hours at 4°C. For rpoSI+DsrA construct, we mixed 2 µl of Cy3-rpoSI (40 µM in H2O), 7 µl of Cy5-DsrA (52 µM in H2O), and 1 µl of NaCl (5 M) and followed the same annealing protocol as used for rpoSI+II.

Fluorescence measurements

Fluorescence emission spectra were measured in T50 buffer using a Varian Eclipse fluorospectrophotometer. While the sample was excited at 500 nm, fluorescence emission for Cy3, Cy5 and Cy5.5 were collected at 565, 670 and 702 nm, respectively. To increase the stability of RNA secondary structures and mimic the low temperatures that might be prevalent during effective rpoS translation, all measurements were done at 15°C. Fluorescence kinetic time traces were analysed using SigmaPlot (Systat) and Origin (Microcal).

Electro-mobility shift assay (EMSA)

The bandshift assay was performed with the same concentrations of labelled RNA and Hfq as described for FRET studies. After incubation, samples were loaded onto a 0.75 mm thick, 20% polyacrylamide (29:1) gel containing 150 mM Tris-HCl (pH 8.0). The gel was subjected to electrophoresis using a Mini-PROTEAN (Bio-Rad) apparatus in 25 mM Tris base and 250 mM glycine at 20°C for 4 h at 70 V. The gels were scanned using a Typhoon imager (Amersham Biosciences, USA).

For quantification of annealed and melted RNA, we analysed the Cy3 label using the ImageJ software (http://rsb.info.nih.gov/ij/).

Purification of Hfq

Hfq was purified from the BL21(DE3) E. coli strain transformed with the plasmid pTE607 as previously described (33). UV absorption spectrum of Hfq indicated that Hfq is RNA and ATP free.

RESULTS

RNA constructs for FRET studies

To investigate how Hfq affects the interaction between the DsrA (sRNA) and its target site on the rpoS (mRNA), we reduced the size of the interacting RNAs to three short fragments of 37 nt each containing all the sequences involved in intra- and intermolecular base pairing governing the translational regulation of rpoS (Figure 2a). The DsrA fragment was labelled with Cy5 (acceptor I) and a rpoS fragment, containing sequences upstream of the ribosome-binding site which is partially complementary to DsrA, was labelled with Cy3 (donor); they are referred to as Cy5-DsrA and Cy3-rpoSI, respectively. The third fragment containing the Shine-Dalgarorno sequence and the start codon of rpoS harbours a Cy5.5 label (acceptor II) and is named Cy5.5-rpoSI. Annealing of Cy3-rpoSI and Cy5.5-rpoSI mimics the stem of the secondary structure that masks the Shine-Dalgarorno sequence of rpoS. All three RNA fragments were labelled at their 5′ or 3′ extremities so that annealing of Cy5-DsrA to Cy3-rpoSI and Cy5.5-rpoSI to Cy3-rpoSI results in high FRET (see Methods and Figure 1).

Hfq stimulates the annealing of rpoS and DsrA

To test whether Hfq promotes DsrA…rpoS annealing (Figure 2a), we prepared 25 nM of Cy3-rpoSI and 50 nM of Cy5-DsrA in T50 buffer at 15°C. In the absence of Hfq, the emission spectrum showed negligible Cy5 signal (Figure 2b, black line), which did not change significantly for 5 min (Figure 2c), indicating that spontaneous annealing is slow under these conditions. We did observe significant spontaneous annealing at higher temperatures and higher RNA concentrations (Supplementary Figure S1). Addition of Hfq (28 nM in hexamer) at t = 9.5 min caused an abrupt increase in the Cy5 signal and a corresponding decrease in the Cy3 signal, indicating that Hfq facilitates rapid association of Cy5-DsrA and Cy3-rpoSI (Figure 2c). This large increase in FRET, which occurred in less than 20 s, was followed by a more gradual increase in FRET (lifetime of ~9 min). The emission spectrum obtained 25 min after Hfq addition (Figure 2b, blue line) also shows a clear increase in the Cy5 signal and a decrease in the Cy3 signal. This result was confirmed by electromobility shift assay (EMSA) which showed that 15% of rpoSI is in the annealed form after the first 5 min with the reaction proceeding further with a lifetime of >10 min, similar to the slow phase in the FRET data (Figure 2d and e). The Cy5-DsrA lane shows an additional...
Addition of 28 nM Hfq to 25 nM rpoSI+DsrA (in T50; 15°C) has two clear effects on the fluorescence signals (Figure 3b). First, we observed an abrupt increase in both Cy3 and Cy5 signals upon the addition of Hfq. The increase in both fluorescence signals, typically 20–30% (Supplementary Figure S2a), reflects an interaction of the protein with the cyanine dyes which blocks a non-radiative decay pathway (34,35). We cannot distinguish whether this effect is due to Hfq binding to the individual strands or to the annealed rpoSI+DsrA since the EMSA experiments suggest that there is a substantial fraction of RNA in the single-stranded form even after thermal annealing (Figure 3e). Second, the abrupt increase of fluorescence is followed by a gradual decrease in the Cy5 signal and an accompanying increase in the Cy3 signal, which indicates that Hfq melts rpoSI+DsrA over a time scale of minutes. The lifetime of FRET signal change decreased with increasing Hfq concentration and was ~4 min at saturation (560 nM Hfq), (Figure 3c). The melting activity of Hfq was enhanced with increase in temperature with the lifetime of 1.7 min at 37°C for 56 nM Hfq (Figure 3d). Spontaneous melting of the rpoSI+DsrA duplex in the absence of Hfq was not observed at our standard temperature of 15°C but was observed at 37°C and was about 4-fold slower than the Hfq-induced melting (Figure 3d). This disruption of rpoSI+DsrA duplex by Hfq over a time scale of minutes was also confirmed by EMSA performed under the same conditions (Figure 3e and f). In contrast, we found that the melting of DsrA loop I domain by Hfq to be extremely slow even under saturating conditions (up to 2 μM Hfq; Kd = 320 nM) with lifetimes >30 min (data not shown), suggesting that melting of rpoSI+DsrA by Hfq is a specific activity of the protein rather than a result of non-specific binding of the protein to RNA duplexes.

Hfq melts the rpoSI+DsrA duplex

We examined if Hfq has the ability to disrupt the rpoS stem structure which would enhance the eventual annealing with DsrA (Figure 4a). For this measurement, we annealed Cy3-rpoSI and Cy5-DsrA as described in Methods. The annealed RNA complex, named rpoSI+II, mimics the secondary structure of rpoS. Before adding Hfq, the emission spectrum shows significant Cy5.5 signal (Figure 4b, black line) due to intact rpoSI+II (25 nM in T50 at 15°C). Cy3 and Cy5.5 signals did not change significantly for 5 min (Figure 4c) indicating that rpoSI+II is stable under these conditions. However, when 28 nM Hfq was added, the Cy5.5 signal gradually decreased with a concomitant Cy3 signal increase over a time scale of minutes (Figure 4c). The emission spectrum obtained 25 min after Hfq addition (Figure 4b, blue line) also shows a clear decrease in FRET. These results demonstrate that Hfq can induce melting of the rpoS stem, independent of DsrA. The average melting time obtained by fitting the FRET decrease to an exponential decay decreases with Hfq concentration and begins to saturate at about 4 min at concentrations above 300 nM Hfq (Figure 4d). This saturation is consistent with the formation of a complex between Hfq and rpoS with larger molecular-weight species that we attribute to the DsrA dimer as reported previously (13). Based on these data, we interpret the rapid FRET increase as the ternary complex formation via simultaneous binding of Hfq to rpoSI and DsrA and assign the subsequent slow FRET increase to the eventual annealing of the two RNA molecules. A similar ternary complex has been reported previously with the annealing reaction, i.e. melting of the rpoS and DsrA (13).

Hfq helps annealing of DsrA and rpoS. (A) Scheme to examine the annealing of DsrA and rpoS mediated by Hfq. (B) Emission spectra of 25 nM Cy3-rpoSI and 50 nM Cy5-DsrA in T50 buffer without Hfq or after incubating the sample for 10 min without Hfq (black line), and then with 28 nM of Hfq (blue line). (C) Emission intensity time trace of Cy3 at 565 nm (green trace) and Cy5 at 667 nm (red trace). Hfq (28 nM) added at 10 min results in an abrupt increase (~20%) in FRET followed by a slow increase (average annealing time = 9 min). (D) EMSA experiment confirms the annealing reaction. Green and red bands correspond to the fluorescence scans with Cy3 and Cy5 filters respectively. Lane 1: Cy3-rpoSI and Cy5-DsrA (50 nM) in the absence of Hfq, Lanes 2–4: Cy3-rpoSI and Cy5-DsrA in the presence of Hfq (28 nM) after incubation for 5, 10 and 15 min at 15°C. Lane 5: Cy5-DsrA and Lane 6: Cy3-rpoSI. (E) The amount of Cy3-RNA in the annealed form (indicated in the gel image) is quantified and shown in the plot.

Figure 2. Hfq helps annealing of DsrA and rpoS. (A) Scheme to examine the annealing of DsrA and rpoS mediated by Hfq. (B) Emission spectra of 25 nM Cy3-rpoSI and 50 nM Cy5-DsrA in T50 buffer without Hfq or after incubating the sample for 10 min without Hfq (black line), and then with 28 nM of Hfq (blue line). (C) Emission intensity time trace of Cy3 at 565 nm (green trace) and Cy5 at 667 nm (red trace). Hfq (28 nM) added at 10 min results in an abrupt increase (~20%) in FRET followed by a slow increase (average annealing time = 9 min). (D) EMSA experiment confirms the annealing reaction. Green and red bands correspond to the fluorescence scans with Cy3 and Cy5 filters respectively. Lane 1: Cy3-rpoSI and Cy5-DsrA (50 nM) in the absence of Hfq, Lanes 2–4: Cy3-rpoSI and Cy5-DsrA in the presence of Hfq (28 nM) after incubation for 5, 10 and 15 min at 15°C. Lane 5: Cy5-DsrA and Lane 6: Cy3-rpoSI. (E) The amount of Cy3-RNA in the annealed form (indicated in the gel image) is quantified and shown in the plot.

We examined if Hfq can promote the reverse reaction, i.e. melting of the rpoSI-DsrA duplex (Figure 3a). We pre-annealed Cy3-rpoSI and Cy5-DsrA (See Methods for details), termed here as rpoSI+DsrA.
a $K_d$ around 150 nM as shown by previous affinity measurements (13) and our anisotropy measurements (Supplementary Figure S2b). Therefore, binding of Hfq to $rpoS$ is slow, taking many minutes, under our standard conditions (28 nM Hfq hexamer) and hence the slower melting observed in Figure 4c.

**Hfq does not anneal the $rpoS$ stem**

In contrast to Hfq’s ability to promote $rpoSI$...DsRA annealing, we did not observe any increase in FRET when Cy3-$rpoSI$ and Cy5.5-$rpoSI$II were incubated in the presence of Hfq under the same condition (Figure 4e and f), despite the fact that Hfq is able to bind both $rpoSI$ and $rpoSI$II as observed by EMSA and anisotropy measurements (Supplementary Figure S2c and b). For this measurement, we incubated 25 nM Cy3-$rpoSI$ and 62.5 nM Cy5.5-$rpoSI$II at 15°C for 25 min without Hfq (black curve) and with 28 nM of Hfq (blue curve). Therefore, the annealing activity of Hfq is specific to DsRA and $rpoSI$.

**Strand exchange reaction**

We then probed the effect of Hfq on the interaction between DsRA and $rpoSI$ ($rpoSI$+II) as shown schematically in Figure 5a. First, we mixed 25 nM $rpoSI$+II duplex and 65 nM Cy5-DsRA in T50 buffer at 15°C to investigate whether Cy5-DsRA can anneal by itself to Cy3-$rpoSI$ and release Cy5.5-$rpoSI$II. After 30 min incubation at 15°C, we measured the emission spectrum (Figure 5b). Although Cy3 and Cy5.5 emission is clear, Cy5 signal is negligible, indicating that spontaneous interaction between DsRA and $rpoS$ does not occur effectively under our experimental conditions (black line; Figure 5b). However, incubation with 28 nM Hfq for 25 min results in a decreased Cy5.5 signal and an increase in Cy5 emission (blue line; Figure 5b). A larger change in fluorescence signals is seen with 280 nM Hfq (Figure 5c). The data show that Hfq enhances both the disruption of $rpoS$ internal structure and annealing of DsRA with the upstream strand of the $rpoSI$ stem, thus in effect carrying out a strand exchange reaction.

Kinetic data on the strand exchange reaction provide additional details (Figure 5d and e). When Hfq was added to a mixture of DsRA and $rpoSI$+II at $t = 5$ min, we observed an abrupt decrease in Cy3 signal and a simultaneous increase in Cy5 signal which we attribute to the rapid association of free single-stranded DsRA and $rpoSI$ as we have shown in Figure 2c. This was followed by a slow decrease in the Cy5.5 signal and a slow increase in the Cy5 signal without significant changes in the Cy3 signal indicating that DsRA gradually replaces $rpoSI$II to pair up with $rpoSI$. The decay lifetime of the Cy5.5 signal decrease is ~13 min, similar to the time scale of melting of $rpoSI$+II at the same Hfq concentration. Figure 5e shows fluorescence signals versus time from $rpoSI$+II where Hfq (28.5 nM) is added at $t = 5$ min followed by DsRA addition at $t = 20$ min. Slow melting of $rpoSI$+II was observed upon Hfq addition similar to what has been seen in Figure 4c. When DsRA was added, we observed a very rapid increase in FRET from Cy3 to Cy5.5 followed by a slower FRET increase as we have seen from the experiment using $rpoSI$ and DsRA only. This experiment suggests that the strand exchange reaction can also proceed by slow melting of the $rpoS$ duplex followed by rapid association of DsRA to melted $rpoS$.

**DISCUSSION**

We have examined how Hfq affects the interaction between a non-coding sRNA, DsRA and its regulation target mRNA, $rpoS$ using real-time FRET assays. In order to focus on the specific parts of the system that...
are involved in translational regulation of rpoS by DsrA, we limited our investigation to small fragments of DsrA and rpoS. In particular, we omitted the P3 site of rpoS and loop II domain in DsrA both of which contact Hfq strongly according to footprinting experiments (13). However, our constructs conserved all the known intra- and inter-strand base-pairing as well as uridine-rich regions important for Hfq binding (13,16).

We demonstrated that Hfq assists in annealing of pre-melted DsrA and rpoS. FRET measurements showed a rapid FRET increase within a few seconds after Hfq addition due to the binding of both DsrA and rpoS to Hfq and formation of a partially annealed complex (allowing energy transfer between the RNAs that are in close proximity). This rapid binding step is followed by a slower FRET increase signifying the subsequent RNA annealing. This is consistent with previous observations of a partially annealed intermediate DsrA…rpoS complex and only two fold enhancements in annealing in the presence of Hfq at 30°C (13) and our own EMSA studies. This implies that actual rates of Hfq-mediated annealing are much slower than RNA binding and are in the time scale of minutes at low temperatures.

Our real-time FRET analysis also showed that Hfq melts rpoS+II complex over several minutes. This activity could be important in promoting the final strand exchange since annealing of DsrA and rpoS requires pre-melting of the rpoS secondary structure. This observation is contrary to previous nuclease footprinting experiments which reported that Hfq recognized several sites on the

Figure 4. Hfq melts the stem structure of rpoS but does not promote annealing. (A) Scheme to show Hfq assisted melting of rpoS. (B) Emission spectra of 25 nM rpoS+II or after incubating the sample for 5 min without Hfq (black line), and then for 25 min with 28 nM Hfq (blue line). (C) Intensity time trace of Cy3 (green lines) and Cy5.5 (red purple lines); Hfq (28 nM) was added at 5 min. Addition of Hfq results in gradual FRET decrease (average lifetime ~15 min). (D) Average melting times were measured as a function of Hfq concentration (as reported that Hfq recognized several sites on the

Figure 5. Strand exchange reaction promoted by Hfq. (A) Schematic of the strand exchange reaction mediated by Hfq. (B) Emission spectra of the mixture of 25 nM of the rpoS+II and 62.5 nM of Cy5-DsrA in T50 buffer without Hfq. Comparison of the emission spectra of the mixture 15 min after adding 28 nM Hfq (blue lines) and before adding Hfq (black lines) shows a shift in emission spectrum from Cy5.5 (707 nm) to Cy5 (667 nm). (C) Comparison of the emission spectra before (black line) and after addition of 282 nM Hfq (blue line). (D) Intensity time traces at Cy3, Cy5 and Cy5.5 emission wavelengths where 28 nM Hfq is added at t=5 min to a solution containing rpoS+II and Cy5-DsrA. (E) Intensity times traces at Cy3, Cy5 and Cy5.5 emission wavelengths where Hfq is added at t=5 min and Cy5-DsrA is added at t=20 min to a solution containing rpoS+II.
CONCLUSION

Our results confirm that Hfq rapidly associates with small RNA regulator, DsrA, and its target rpoS mRNA simultaneously increasing their local concentration. We also show that Hfq is a chaperone that can change the conformation of rpoS making it accessible to DsrA. We suggest that Hfq accelerates the arrival of the dynamic equilibrium between annealing and dissociation of RNA strands, and spectroscopic assays described here were able to track these processes in real time. Two primary results must be emphasized: (i) Hfq dramatically accelerates the association of DsrA and rpoS but eventual annealing of DsrA…rpoS is slower and (ii) Hfq melts the rpoSI+II complex over several minutes. Taking these results into account, we propose a model for the function of Hfq in the translational regulation of rpoS by DsrA (Figure 6). Hfq binds rapidly to both rpoS and DsrA and melts the stem of rpoS (average reaction time ~4 min), which appears to be irreversible. Increased local concentration of both RNAs and melting of rpoS facilitates the annealing between rpoS and DsrA (average reaction time ~9 min) (13). Finally, Hfq dissociates from RNA duplex due to its low binding affinity (13). We have also shown that at least one step in the reaction is reversible so that Hfq facilitates the melting of DsrA and rpoS (average reaction time ~4 min). Such an activity of Hfq may be useful in the reversal of post-transcriptional regulation (for example, by re-sequestering the Shine–Dalgarno sequence and blocking the ribosome binding site). The overall process is rather slow and it may be that additional co-factors might be assisting this translational regulatory machinery in vivo.

ACKNOWLEDGEMENTS

This work was supported by CNRS (UPR 9073), University Denis Diderot-Paris 7 and by the National...
REFERENCES

1. Franze de Fernandez, M.T., Hayward, W.S. and August, J.T. (1972) Bacterial proteins required for replication of phage Qβ ribonucleic acid. J. Biol. Chem., 247, 824–821.

2. Vecerek, B., Moll, I. and Blasí, U. (2005) Translational autocontrol of the Escherichia coli hfq RNA chaperone gene. RNA, 11, 976–984.

3. Hajnsdorf, E. and Regnier, P. (2000) Host factor Hfq of Escherichia coli stimulates elongation of poly(A) tails by poly(A)polymerase I. Proc. Natl. Acad. Sci. U.S.A., 97, 1501–1505.

4. Rasmussen, A.A., Eriksen, M., Gilany, K., Udzen, C., Franch, T., Petersen, C. and Valentin-Hansen, P. (2005) Regulation of ompA mRNA stability: the role of a small regulatory RNA in growth phase-dependent control. Mol. Microbiol., 58, 1421–1429.

5. Muffler, A., Traulsen, D.D., Fischer, D., Lange, R. and Hengge-Aronis, R. (1997) The RNA-binding protein HF-1 plays a global regulatory role which is largely, but not exclusively, due to its role in expression of the σ54 subunit of RNA polymerase in Escherichia coli. J. Bacteriol., 179, 297–300.

6. Sledjeski, D.D., Whitman, C. and Zhang, A. (2001) Hfq is necessary for regulation by the untranslated RNA DsrA. J. Bacteriol., 183, 1997–2005.

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

8. Masse, E., Majdalani, N. and Gottesman, S. (2003) Regulatory roles for small RNA in bacteria. Current Opinion in Microbiology, 6, 120–124.

9. Storz, G. (2002) An expanding universe of noncoding RNAs. Science, 296, 1260–1263.

10. 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.

11. Storz, G., Opdyke, J.A. and Zhang, A. (2004) Controlling mRNA stability and translation with small, noncoding RNAs. Curr. Opin. Microbiol., 7, 140–144.

12. Kawamoto, H., Koide, Y., Morita, T. and Aiba, H. (2006) Base-pairing requirement for RNA silencing by a bacterial small RNA and acceleration of duplex formation by Hfq. Mol. Microbiol., 61, 1013–1022.

13. Lease, R.A. and Woodson, S.A. (2004) Cycling of the Sm-like protein Hfq on the DsrA small regulatory RNA. J. Mol. Biol., 344, 1211–1223.

14. 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.

15. 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.

16. Rolle, K., Zywicki, M., Wyszeko, E., Barciszewska, M.Z. and Barciszewski, J. (2006) Evaluation of the dynamic structure of DsrA RNA from E. coli and its functional consequences. J. Biochem. (Tokyo), 139, 431–438.

17. Moll, I., Leitsch, D., Steinhauser, T. and Blasí, U. (2003) RNA chaperone activity of the Sm-like Hfq Protein. EMBO Reports, 4, 284–289.

18. Herschlag, D. (1995) RNA chaperones and the RNA folding problem. J. Biol. Chem., 270, 20871–20874.