Cycling of RNAs on Hfq

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The RNA chaperone Hfq is a key player in small RNA (sRNA)-mediated regulation of target mRNAs in many bacteria. The absence of this protein causes pleiotropic phenotypes such as impaired stress regulation and, occasionally, loss of virulence. Hfq promotes rapid sRNA-target mRNA base pairing to allow for fast, adaptive responses. For this to happen, sRNAs and/or mRNAs must be bound by Hfq. However, when the intra- or extracellular environment changes, so does the intracellular RNA pool, and this, in turn, requires a correspondingly rapid change in the pool of Hfq-bound RNAs. Biochemical studies have suggested tight binding of Hfq to many RNAs, indicating very slow dissociation rates. In contrast, the changing pool of binding-competent RNAs must compete for access to this helper protein in a minute time frame (known response time for regulation). How rapid exchange of RNAs on Hfq in vivo can be reconciled with biochemically stable and very slowly dissociating Hfq-RNA complexes is the topic of this review. Several recent reports suggest that the time scale discrepancy can be resolved by an "active cycling" model: rapid exchange of RNAs on Hfq is not limited by slow intrinsic dissociation rates, but is driven by the concentration of free RNA. Thus, transient binding of competitor RNA to Hfq-RNA complexes increases cycling rates and solves the strong binding/high turnover paradox.

Introduction

Not long ago, adaptive or developmental changes in gene expression—irrespective of organism—were almost exclusively attributed to transcriptional regulation. Today, we know that post-transcriptional control is equally important. The plethora of regulatory RNAs discovered in genome-wide searches some 10 years ago in bacteria, archea and eukaryotes[1-5] has added an additional layer of regulatory complexity. Bacterial small RNAs (sRNAs) are a structurally heterogeneous group of RNAs which, when induced under specific conditions, generally act by base-pairing to mRNA targets to either repress or activate protein expression.[6,7] Most bacteria probably encode hundreds of sRNAs. Similar to their eukaryotic counterparts, the miRNAs, they often have multiple targets and, since transcription factor (TF) mRNAs are often among these, this indirectly extends target space to encompass regulons under the control of the TF in question (e.g., ref. 7). Thus, it has been argued that in, for instance,

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Escherichia coli, about half of the genes may at some point or another be subject to regulation by sRNAs.[8] For this, however, most sRNAs (at least in many Gram-negative bacteria) require a helper protein.

Hfq was discovered almost 50 years ago as an essential co-factor for Qß (RNA bacteriophage) replication.[9] This protein forms homohexameric doughnut-like structures,[10-13] and each monomer carries the signature Sm motif of its eukaryotic counterparts, the heteroheptameric Sm and Sm-like proteins. Akin to these proteins, Hfq is an avid RNA binder, and this property suggests that it is well-equipped for a functional involvement in RNA transactions.

Trans-encoded (only partially target-complementary) bacterial sRNAs often require Hfq for regulatory potency. Hfq can protect RNAs from degradation, promote high sRNA-mRNA association rates, or act as an RNA chaperone to render folded structures open for interaction (for a recent review, see ref. 14). Studies on simple model RNAs additionally demonstrated Hfq's annealing and strand-displacing activities (e.g., refs. 15–17). Not surprisingly, Δhfq strains display pleiotropic phenotypes such as impaired stress responses, population behavior changes, altered metabolic regulation and loss of virulence.[14,18-20] The fraction of genes whose expression is affected by the presence/absence of Hfq differs between bacterial species, but tends to range from 5–25%.[21-24] Though other effects on gene expression are plausible (Hfq interacts with RNA polymerase, Rho factor, poly-A polymerase I, ribosomal protein S1, RNase E, PNPase and others[25-29]), the primary role of this protein is in sRNA-mediated control.[14] In line with this, numerous sRNAs and mRNAs have been found in complex with Hfq in vivo (see below).

A general requirement for all Hfq-related RNA transactions is the need to rapidly exchange binding partners, i.e., to cycle sRNAs and/or mRNAs on the Hfq pool. This review addresses this issue, which arose from paradoxical results: RNA-Hfq complexes have very low intrinsic dissociation rates in vitro, suggesting that cycling should be slow, but newly induced sRNAs promote target effects in vivo within 1–2 min.[30,31] To properly address this problem and its solution, I will give a short background on Hfq: its binding surfaces, RNA binding properties and its effect on RNA-RNA pairing.

Hfq Structures, Binding Surfaces and RNA Binding

Structures of Hfq are covered more extensively elsewhere in this issue. In brief, crystal structures of the core regions of hexameric Hfq from several bacteria and archaea have been published.[10-13,32-35] The Hfq ring structure displays two faces—denoted proximal

and distal—with distinct properties and preferences for specific RNA substrates. The distal face of *E. coli* Hfq avidly binds single-stranded RNA with ARN (A, adenosine; R, purine; N, any nucleotide) motifs; each monomer binds one motif, and up to 18 nt can be accommodated on the hexamer.[13] A co-crystal structure of *Staphylococcus aureus* Hfq shows a U-rich RNA oligo bound on the inner rim of the proximal face, with one nucleotide contact per monomer.[10] More recently, it was reported that *Salmonella typhimurium* Hfq binds U-rich 3' ends of RNAs (e.g., Rho-independent terminators). The specific binding pocket for the 3'-hydroxyl group is located on the proximal face of the Hfq monomers, as shown in a high-resolution crystal structure.[34] 3'-end binding increases affinity and significantly enhances the regulatory efficiency of sRNAs.[36]

The two Hfq faces with their different RNA sequence/nucleotide preferences suggest a scenario in which sRNAs—most often carrying U-rich internal motifs and a U-tailed terminator[37]—preferentially bind the proximal face,[38] whereas mRNA targets—with A-rich motifs often found in 5'-UTRs and ribosome-binding sites[13,39]—are preferentially bound on the distal face. Face preferences have been established for several artificial and natural RNAs, primarily by using mutant Hfq proteins with amino acid changes in critical positions, and by competition assays (e.g., refs. 38, 40 and 41). Though some RNAs show almost exclusive face specificity, others clearly are capable of binding either face, or both simultaneously (e.g., refs. 42–44). Furthermore, a recent study identified the outer rim of Hfq as a third important, and distinct, interaction region for RNAs. Multiple lateral surface contacts of sRNA body sequences, with the 3'-end usually still anchored on the proximal face, were supported by the effects of introduced amino acid changes.[45] This important result indicates that a simple two-face model may not adequately reflect binding modes. Finally, many but not all bacteria encode Hfq with positively charged unstructured C-terminal extensions. Their functional implications are controversial; some labs have observed a requirement for mRNA binding and riboregulation,[41,46,47] others failed to see effects upon deletion of the C-terminal domain.[48]

Several Binding Sites—For What?

The different surface regions on Hfq—proximal, distal, lateral, and perhaps within the C-termini—and their presence in a homohexameric arrangement, probably confer a favorable property for interactions with potentially thousands of sequence- and structure-wise different RNAs. Most single-stranded, unstructured RNA segments bind Hfq, likely at the sites at which their sequence patterns fit surface patches with appropriate specificities. Hence, not surprisingly, a survey of the literature shows that almost any sRNA, mRNA and artificial RNA tested does bind Hfq, often with very high affinity (sub- to mid-nanomolar K_d-values, e.g., refs. 38, 42 and 49). Accordingly, the majority of enterobacterial sRNAs and more than 1,200 mRNA species can be pulled out in Hfq-co-IP experiments,[24,50,51] and similar results have been obtained in other bacteria.[20,52] So how does Hfq binding affect the fate and activities of all these RNAs?

Two effects are well established. The first concerns metabolic stability of bound RNAs. Many sRNAs are unstable in the absence of Hfq (e.g., refs. 51, 53 and 54), likely because they need protection from degradation by RNase E and exoribonucleases;[55] RNase E and Hfq have similar binding preferences for unstructured AU-rich sequences[56] and a 3'-terminal U of a terminator becomes inaccessible in the proximal face pocket of Hfq.[34] The second effect is on sRNA-target mRNA interactions. Biochemical experiments strongly indicate that, in most cases, Hfq increases cognate sRNA-mRNA association rates,[39,42,57] even though stabilization of RNA-RNA duplexes (i.e., a K_d-effect) has been suggested as an alternative explanation.[58]

For fast sRNA-target RNA binding, the two partners have to meet on Hfq, and we assume here one hexamer sandwiched between the two RNAs. Other stoichiometries have been reported[44,59,60] but are omitted from this discussion. High affinity of each RNA alone reflects the sum of the energetic contributions of several monomeric sites, on whatever binding surface that matches the RNA in question. For simultaneous binding of two RNAs, many arrangements are possible (see above). In a certainly oversimplified scenario, based on some in vitro experiments, sRNAs bind to the proximal face, and mRNAs containing $(ARN)_n$ or AAYAA motifs, generally enriched near start and stop codons,[49] to the distal face. Since steric clashes are not predicted when both faces are occupied by RNAs,[13] Hfq can be regarded as the platform on which simultaneously bound RNAs meet. For subsequent base-pairing, the RNA-Hfq contacts should not block the complementary sequences in both RNAs, but rather be in close vicinity.[61,62] Thus, simultaneous binding of two RNAs (e.g., refs. 17, 45, 46 and 63) provides high local concentration of both interactants. Unfolding of RNA structure elements by the RNA chaperone (e.g., refs. 39 and 64), and presentation of extended and flexible RNA segments, would then allow for a sampling of sequence space and the rapid annealing of complementary sequences to form the heteroduplex.[15,17,57,65-67]

Exchanging RNAs on Hfq
In Vitro—The Need for Rapid Cycling

Since sRNAs and mRNAs must meet on Hfq to experience a pairing rate-increasing effect, and the time span from induction of an sRNA to observed major effects on target gene expression is short (1–2 min[30,31]), RNAs must rapidly exchange on Hfq. Given the approximate time frame, one can ask whether the biochemical properties of Hfq can account for in vivo regulation. Many labs have conducted binding studies with Hfq and their favorite sRNA and target mRNA substrates.[34,38-40,42,49,55,60,64,68-71] The dissociation constants (K_d) measured, though varying dependent on the identity of the RNA in question and the experimental conditions, generally ranged from mid- to sub-nM. Hence, binding is very tight, and dissociation rates should therefore be low. If true, this would create problems in an in vivo setting. We can consider, in principle, two conditions. In the first scenario, which by now has good experimental support, binding-competent RNAs are in molar excess over Hfq hexamers, and essentially all Hfq will be RNA-bound (refs. 42, 53 and 72 and
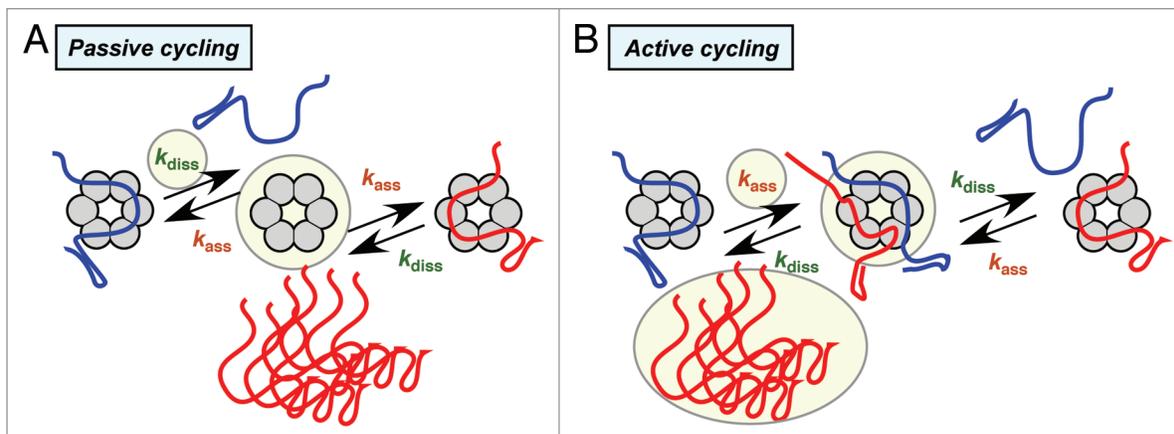
Figure 1. Passive and active cycling models. Resident RNAs (blue) are replaced by competing RNAs (red) on Hfq. Yellow circles highlight important differences. (**A**) Entry of red RNA requires prior dissociation of the Hfq-RNA complex and is limited by the *intrinsic* dissociation rate constant k_{diss}. (**B**) Red RNA binds to RNA-Hfq complex. Cycling rates depend on the concentration of free red RNA and the second order association rate constant k_{ass}. See text for details.

see below). At low Hfq-RNA dissociation rates, newly induced sRNAs (or other RNAs) can only slowly access their share of the Hfq pool and, thus, the fast kinetics of regulation cannot be accounted for. In a second scenario, we can assume instead that Hfq is in excess over binding-competent RNA. If so, nascent sRNAs/target RNAs can bind to the free Hfq pool. However, though solving the problem of Hfq access, other problems arise. For instance, if sRNAs have to meet their target mRNAs on Hfq, a free Hfq pool would decrease the probability of finding the correct RNA partner, since RNAs would statistically be mostly distributed as single RNA-Hfq complexes. Furthermore, Hfq excess would entail sluggish responses upon environmental change. If conditions change during growth or stress responses, new sets of induced sRNAs should replace those that presently occupy Hfq and have become inadequate. The "new" sRNAs would easily find empty Hfq, however, "old" sRNAs would be permitted to linger on and cause misregulation. Thus, I argue that cycling of RNAs on Hfq is needed in scenarios that involve RNA *or* Hfq molar excess. As will be shown below, available data suggest that, at least in enterobacteria, Hfq is indeed saturated with RNA.

RNA Concentration-Driven Exchange of RNAs on Hfq—'Active Cycling'

Even though tight RNA-Hfq binding had been observed in vitro, the paradoxical implications of this took some time to be recognized. Low K_d-values suggest very slow RNA-Hfq dissociation rates. In a conventional "passive" model, a bound RNA must first dissociate before a second RNA can be granted access to the same binding site (**Fig. 1A**). Thus, exchange cannot be faster than the intrinsic Hfq-RNA dissociation rate, irrespective of the concentration of any competing RNA. At low K_d-values, where half-lives may exceed an hour, replacing one RNA with another one would therefore occur on the same time scale, clearly not in accordance with in vivo response times. In light of this inconsistency,

it seemed appropriate to consider alternative models that might account for rapid cycling of RNAs on Hfq.

In principle, simultaneous transient binding of two RNAs should be feasible even on the same face of Hfq. The hexameric character of this protein, and the fact that model RNAs can contact several subunits (even all six) in an equivalent fashion,[13] suggests a way by which RNA exchange could be driven by the free RNA concentration. In fact, early studies of the hexameric termination factor Rho suggested RNA-driven displacement of bound RNA substrates.[73,74] Hence, if this were to apply to Hfq, release of resident RNAs would follow second-order rather than first-order kinetics. Initial observations suggested this,[75] and strong support for such an "active cycling" model (**Fig. 1B**) has since been obtained.[38,42,43] The key experiments in these three papers used filter binding experiments and/or surface plasmon resonance. This is important since a clear-cut effect of added competitor RNA on dissociation rates of preformed Hfq-RNA complexes is difficult if not impossible to quantify by traditional gelshift assays; intrinsic (no free RNA added) dissociation rate constants need to be measured by very high dilution of the complex. The results in references 38, 42 and 43 collectively demonstrated that intrinsic dissociation of RNA-Hfq complexes is very slow, often at half-lives of > 150 min. This corresponds to k_d-values of 1–4 $\times 10^{-4}$ s^{-1} for various sRNA- or mRNA-Hfq complexes.[38,42] Upon addition of competitor RNA, the dissociation rate of the resident RNA was vastly increased. The competitive potency varied with respect to the identity of the competitor and the resident RNA (see below), but, strikingly, almost all tested sRNAs and mRNAs substantially increased the dissociation rate of any resident RNA (sRNA or mRNA). Titration experiments showed that the dissociation rate increased as a function of competitor concentration. Already at far below µM competitor RNA concentration, half-lives of Hfq-RNA complexes dropped from > 150 min to 1–5 min,[42] thus reconciling in vitro biochemistry with the in vivo time frame of regulation. Importantly, the observed second-order kinetics (association rate constant $\approx 10^6$ M^{-1}s^{-1} [42,43]) implies the
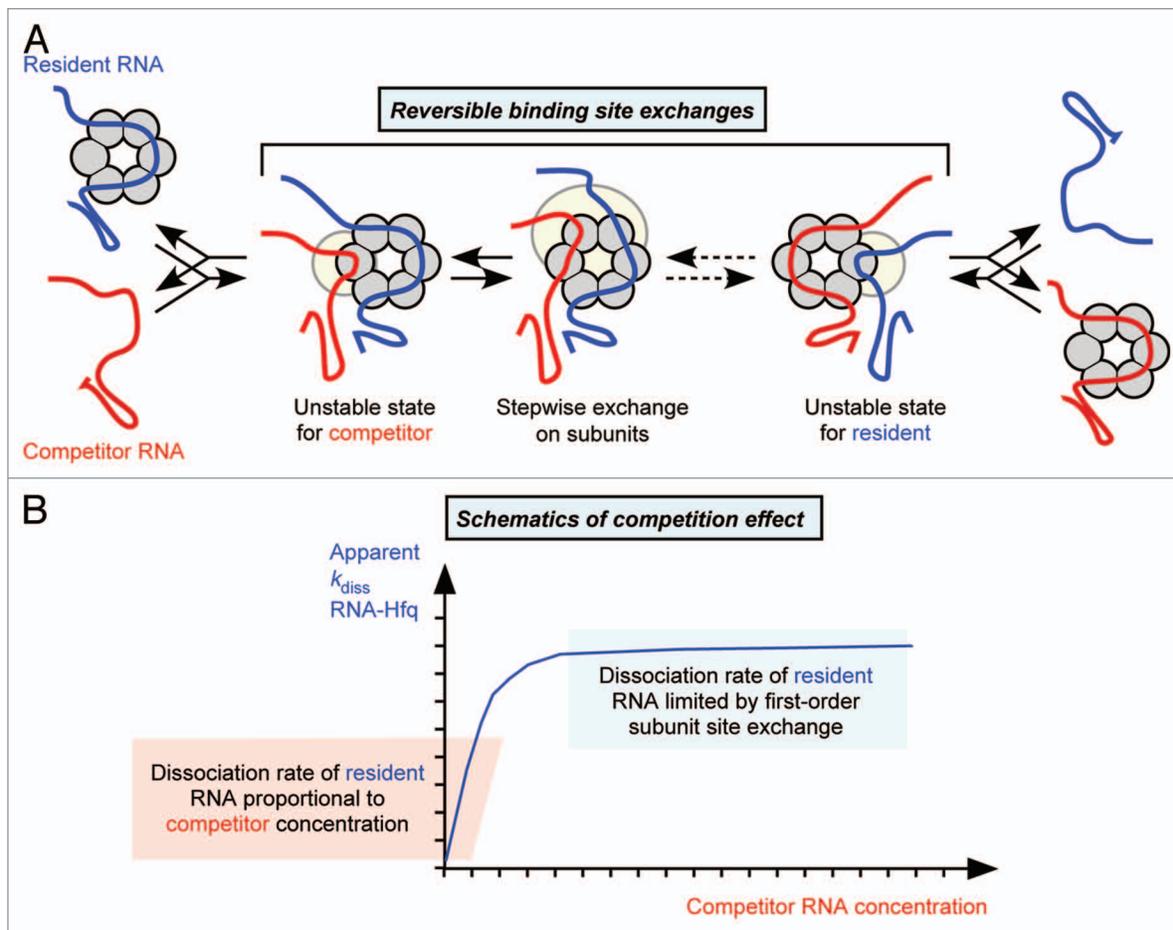
Figure 2. Reversible rearrangement rates limit cycling rates when free RNA is abundant. (**A**) Pathway of replacement of resident (blue) by competitor RNA (red) on Hfq. The picture highlights the strict reversibility and indicates intermediate steps. Dissociation of either RNA is assumed to occur from a one subunit-bound state (yellow circles, left and right). Middle yellow circle: one of several states in which RNAs swop monomeric-binding sites; subsequent states implied by dashed arrows. (**B**) Schematic graph showing the competitor RNA dependence of the dissociation of resident RNA from Hfq. In the lower range, the apparent dissociation rate constant k_{diss} increases as a function of competitor RNA concentration. On the plateau, where binding sites are saturated, first-order rearrangement kinetics becomes limiting. See text for details.

transient formation of Hfq complexes with two RNAs as shown in **Figure 1B**.

The important features of the active cycling model can be summarized as follows: RNAs residing on Hfq are on average in simultaneous contact with several subunits. A free competitor RNA initially contacts single unoccupied subunits on the same Hfq face. Throughout a series of reversible replacement steps, single subunit binding sites are swopped between RNAs. These steps do not require external energy[42,76] and likely involve very small $\Delta\Delta G^o$ values since incremental contributions of RNA elements to subunit binding are similar. Rapid dissociation of one, or the other, RNA occurs from an unstable one-monomer-bound state that probably only involves one remaining RNA-Hfq subunit contact (**Fig. 2A**); this is a rare occupancy state, explaining the low apparent k_d-values. Experiments combined with mathematical modeling showed, in addition, that cycling rates initially are a function of competitor concentration but, at saturation, become limited by the first-order rearrangement rates[42] (**Fig. 2B**). For many competitor RNAs already at ≈100 nM concentration,

corresponding to merely ≈100 molecules in an *E. coli* cell, cycling is pushed into a minute time frame. In other words, the model explains how changes in the population of RNA-Hfq complexes rapidly follow the changes in the cellular RNA pool.

From Simple to More Complicated

Even though active cycling explains RNA exchange on Hfq in general, quantitative variations have been observed. For instance, some RNAs compete better than others with a given Hfq-bound RNA.[38,42] Poor competitors may be easy to interpret. IstR-1 is an sRNA that counteracts SOS-induced toxicity,[77] and does not require Hfq (C. Unoson, unpublished). Accordingly, it is a poor Hfq binder and fails to promote dissociation of bound RNAs[42] (though see ref. 38 for a partially conflicting result). In some cases, face preferences come into play. Poly(A) and *ompF* mRNA cannot displace the Hfq-bound sRNA MicA, but compete efficiently with Hfq-bound *ompA* mRNA. This suggests that poly (A) and *ompF* have an almost exclusive binding preference for

the distal face; *ompA* is preferentially bound on the distal, but MicA on the proximal face (A. Fender, unpublished). Strikingly though, many RNAs in the set tested in ref. 42 displaced RNAs from either face. This can be rationalized by non-exclusive face preferences; experiments with Hfq face mutants showed that binding of a given RNA is rarely abolished, but often weakened (e.g., refs. 38 and 40). From this, we tentatively conclude that competition occurs primarily on the same face, as shown schematically in **Figure 2A**. The wide range of competition-proficient RNAs that displace both distal and proximal face-bound RNAs[38,42] suggests that most RNAs have face-preferences but can occupy additional binding surfaces when the preferred sites are unavailable. For RNAs that may wrap around to simultaneously contact both faces, competition could be more complex.[41,43] Also, the recent reports on sRNA binding to lateral surfaces,[45] and the anchoring of 3'-ends,[34] suggests additional paths by which binding and competition is accomplished, and may explain why the competitive power of an RNA does not simply correlate with its binding affinity (see below).

What happens after sRNAs and mRNAs pair on Hfq? It has been suggested that ternary complexes with base-paired RNAs are unstable, and that Hfq is rapidly released,[59] which would facilitate cycling. However, OxyS-*fhlA*-Hfq ternary complexes are stable (K_d-value of ≈ 20 nM[43]), as are RyhB-*sodB*-Hfq complexes, from which the RyhB-*sodB* pair can be chased by addition of free *sodB* RNA.[75] Intrinsic dissociation of the MicA-*ompA* pair from Hfq is very slow (half-life > 250 min[42]) but again can be chased by several RNAs. It is reasonable to assume that many if not most ternary complexes are stable because falling off requires detachment of the RNA pair from at least two binding surfaces. All this suggests that RNA concentration not only drives dissociation of binary, but also ternary RNA-Hfq complexes.

A recent paper reported results which potentially have implications on binding and cycling. Hfq hexamers are suggested to be surprisingly unstable in vitro, with subunits in dynamic exchange and a monomer-hexamer equilibrium constant of ≈ 0.8 μM.[78] This seems at odds with Hfq-RNA binding at low K_d-values[38,42,43] when using Hfq concentrations of 5–10 nM[42,43] or sub-nM,[38] i.e., conditions where Hfq should be almost exclusively monomeric.[78] Similarly, in single molecule FRET experiments, RNA-RNA dissociation and annealing was measured at 2–10 nM Hfq concentrations.[17] Hfq monomer-RNA complexes have, to my knowledge, never been detected, and should to be too unstable based on the energetics of subunit-RNA interactions. Whether RNA-mediated stabilization of the hexameric state can account for the observed stability of complexes at very low Hfq concentrations needs to be assessed.

RNA Binding, Cycling and Competition on Hfq In Vivo

Moving from the test tube to the living cell, we encounter a paucity of data. In vivo Hfq concentrations are somewhat uncertain. Reported estimates in *E. coli* range from 400–10,000 hexamers per cell (about 0.4–10 μM).[79-81] Co-IP studies, followed by profiling of the Hfq-bound RNAs, have uncovered numerous sRNAs,

mRNAs and other RNAs.[20,24,50-52,82] Collectively, the vast number of different RNAs bound, and a back-of-the-envelope calculation of their approximate copy number, suggests that binding-competent RNAs are in molar excess over Hfq, and that there is no substantial free Hfq pool. Moreover, saturation of Hfq is experimentally supported by two in vivo studies.[53,72] For instance, the magnitude of sRNA-mediated repression was enhanced by overexpression of Hfq, suggesting that this protein is limiting under wild-type conditions.[72] Also, rifampicin run-out experiments in wild-type cells often show biphasic decay kinetics for sRNAs (unpublished); the two phases tentatively suggest one Hfq-bound fraction (protected, slow decay) and one unable to access Hfq (unprotected, fast decay).

Exchange of RNAs on the cellular Hfq pool has been demonstrated. For instance, profiling showed extensive changes in the composition of the Hfq-bound RNA pool upon ectopic overexpression of a single sRNA[83] and during bacterial growth.[51] In a different approach, Moon and Gottesman[53] addressed competition between sRNAs for limiting Hfq. Overexpression of a given sRNA impacted on the effect of other Hfq-dependent sRNAs, measured as effects on expression of their targets. The same conclusion has been reached in ref. 72. This implies replacement of resident sRNAs by induction of a competitor, i.e., cycling. Both approaches[51,53] also suggest that competition and exchange of sRNAs is not always proportional to the RNAs' binding affinity to Hfq, in line with, e.g. ref. 38. Unfortunately, so far, quantitative assessments of cycling rates in vivo appear hard to come by, and time-resolved analyses of RNA changes on the Hfq pool are urgently needed. In qualitative terms, effects of sRNA induction on activity and/or stability on other RNAs suggest favorable and unwanted consequences. On the one hand, replacing previously needed sRNAs with those that are currently in use generates faster adaptive responses, in particular, since most "ejected" sRNAs experience accelerated decay. On the downside, off-target effects, and misregulation of other sRNA regulons is a possibility.[72]

A further twist is suggested by Hussein and Lim.[72] They found that overexpression of unmatched sRNAs and target RNAs as competitors partially decreases the regulatory effect of a different sRNA-mRNA pair, measured by output from a GFP reporter. Matched competitor pairs gave smaller effects. This was interpreted as an accelerated liberation of Hfq upon competitor sRNA-target RNA pairing, leading to higher free Hfq concentrations and, thus, availability for the sRNA that silenced the target fusion gene. For unmatched competitors, longer dwelling times and, thus, Hfq sequestration was assumed. Though it is intriguing that partnering on Hfq may increase cycling, it would be useful to assess the levels of the competitor RNAs on and off Hfq directly, under the same conditions. This might clarify whether the different effects with matched and unmatched competitor pairs primarily stem from altered dissociation rates or different competitor concentrations; coupled degradation of paired RNAs has been reported[31,94] and might be relevant here.

Open Questions and Conclusions

The last years have taught us about the importance of Hfq for sRNA-target RNA regulation, and biochemical experiments have

provided a framework for how Hfq binds to and affects bound RNAs. In this review, much has obviously been simplified, and it is clear that recent discoveries have not yet been adequately incorporated in how we conceptualize Hfq-RNA transactions. For instance, a two-face model cannot properly account for the available binding surfaces on Hfq. Taking into account lateral binding and effects that depend on C-terminal extensions may explain why binding and competition effects can differ. Biochemical studies, perhaps using single-molecule methods,[17] and preferably employing natural sRNA and mRNA substrates rather than short oligo-RNAs, may be useful to study Hfq activities. In particular, a major challenge lies in mapping of contact points on Hfq throughout the binding of an RNA and during the steps of cycling.

The realization that cycling of RNAs is an important aspect of Hfq's global effects on regulation is a step forward. However, in vivo studies that critically test the quantitative aspects known from in vitro cycling experiments are not yet available. The paucity of spatial and temporal information especially limits our understanding of Hfq-RNA binding, cycling and sRNA-target RNA interactions in the cell. Since most results are based on bulk experiments, we have little knowledge of where Hfq and RNAs meet. For instance, RNAs and proteins can have specific intracellular localization. Some bacterial mRNAs have been postulated to crowd near their genes,[84] and Hfq may be either predominantly localized near the cellular membrane or distributed throughout the cell (e.g., refs. 85 and 86). In all experiments in which parameters are averaged over a bulk population, the effects of specific localization of RNAs and/or Hfq are hidden and not resolved. Similarly, transcriptional bursting can lead to significant temporal cell-to-cell differences in concentrations of sRNA and target, and may generate significant noise.[87] Thus, if cells differ significantly in their RNA pool over time, stochastic effects should affect Hfq binding and competition patterns, i.e., cycling, but will stay under the radar in most experiments.

Cycling of RNAs on Hfq must also impact on patterns of sRNA-dependent multi-target regulation. Theory predicts that target effects occur hierarchally,[88] which should reflect both relative sRNA-target RNA association but also Hfq occupancy of the relevant RNAs. Since sRNAs and mRNAs are not statistically acquired by Hfq, but predominantly will enter through competition, cycling kinetics must affect the overall pattern of target expression upon sRNA induction.

Another elusive question concerns why some RNAs do not require Hfq for regulatory efficiency. For instance, plasmid-encoded antisense RNAs are Hfq-independent (ref. 89 and unpublished), perhaps because of their high association rate constants,[90] which permit close to diffusion-limited binding. In contrast, many *trans*-encoded sRNAs display 10–100-fold lower association rate constants and, therefore, may need Hfq. In other bacteria such as, e.g., *Staphylococcus aureus*, Hfq is present but appears to have no impact on sRNA regulation.[91] It is even more puzzling that a truncated variant of RyhB in *E. coli* has lost its Hfq requirement for stability as well as regulation.[92] What this tells us is yet unclear, but the experiment highlights our incomplete understanding of Hfq dependence. So, how does RNA-mediated regulation work in bacteria that lack Hfq altogether? It is possible that alternative proteins (e.g., ref. 93) can step in as functional analogs. Alternatively, selection may have driven the evolution toward fast kinetics RNA-RNA binding pathways in these bacteria, as is the case for Hfq-independent antisense-target RNA pairs in *E. coli* (CopA/T, RNAI/II, IstR1-*tisB*[90]).

In summary, Hfq is a key player in sRNA-target RNA interactions, and beyond. Its major impact on global regulation, ultimately affecting traits important for fitness, is based on its many interactions with RNA molecules. This, and the issue of cycling of RNAs on Hfq discussed in this review, has highlighted the dynamic and complex web of changing interactions that underlie adaptive responses. The properties of Hfq eluded to here are reminiscent of other multimeric RNA-binding proteins in bacteria (e.g., Rho[73,74]), and may well provide a framework for an understanding of Lsm proteins in eukaryotes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science 2001; 294:858-62; PMID:11679671; http://dx.doi.org/10.1126/science.1065062.

2. Lee RC, Ambros V. An extensive class of small RNAs in Caenorhabditis elegans. Science 2001; 294:862-4; PMID:11679672; http://dx.doi.org/10.1126/science.1065329.

3. Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science 2001; 294:853-8; PMID:11679670; http://dx.doi.org/10.1126/science.1064921.

4. Argaman L, Hershberg R, Vogel J, Bejerano G, Wagner EGH, Margalit H, et al. Novel small RNA-encoding genes in the intergenic regions of Escherichia coli. Curr Biol 2001; 11:941-50; PMID:11448770; http://dx.doi.org/10.1016/S0960-9822(01)00270-6.

5. Wassarman KM, Repoila F, Rosenow C, Storz G, Gottesman S. Identification of novel small RNAs using comparative genomics and microarrays. Genes Dev 2001; 15:1637-51; PMID:11445539; http://dx.doi.org/10.1101/gad.901001.

6. Waters LS, Storz G. Regulatory RNAs in bacteria. Cell 2009; 136:615-28; PMID:19239884; http://dx.doi.org/10.1016/j.cell.2009.01.043.

7. Storz G, Vogel J, Wassarman KM. Regulation by small RNAs in bacteria: expanding frontiers. Mol Cell 2011; 43:880-91; PMID:21925377; http://dx.doi.org/10.1016/j.molcel.2011.08.022.

8. Holmqvist E, Unoson C, Reimegård J, Wagner EGH. A mixed double negative feedback loop between the sRNA MicF and the global regulator Lrp. Mol Microbiol 2012; 84:414-27; PMID:22324810; http://dx.doi.org/10.1111/j.1365-2958.2012.07994.x.

9. Franze de Fernandez MT, Eoyang L, August JT. Factor fraction required for the synthesis of bacteriophage Qbeta-RNA. Nature 1968; 219:588-90; PMID:4874917; http://dx.doi.org/10.1038/219588a0.

10. Schumacher MA, Pearson RF, Møller T, Valentin-Hansen P, Brennan RG. Structures of the pleiotropic translational regulator Hfq and an Hfq-RNA complex: a bacterial Sm-like protein. EMBO J 2002; 21:3546-56; PMID:12093755; http://dx.doi.org/10.1093/emboj/cdf322.

11. Sauter C, Basquin J, Suck D. Sm-like proteins in Eubacteria: the crystal structure of the Hfq protein from Escherichia coli. Nucleic Acids Res 2003; 31:4091-8; PMID:12853626; http://dx.doi.org/10.1093/nar/gkg480.

12. Nikulin A, Stolboushkina E, Perederina A, Vassilieva I, Blaesi U, Moll I, et al. Structure of Pseudomonas aeruginosa Hfq protein. Acta Crystallogr D Biol Crystallogr 2005; 61:141-6; PMID:15681864; http://dx.doi.org/10.1107/S0907444904030008.

13. Link TM, Valentin-Hansen P, Brennan RG. Structure of Escherichia coli Hfq bound to polyriboadenylate RNA. Proc Natl Acad Sci USA 2009; 106:19292-7; PMID:19889981; http://dx.doi.org/10.1073/pnas.0908744106.

14. Vogel J, Luisi BF. Hfq and its constellation of RNA. Nat Rev Microbiol 2011; 9:578-89; PMID:21760622; http://dx.doi.org/10.1038/nrmicro2615.

15. Doetsch M, Stampfl S, Fürtig B, Beich-Frandsen M, Saxena K, Lybecker M, et al. Study of E. coli Hfq's RNA annealing acceleration and duplex destabilization activities using substrates with different GC-contents. Nucleic Acids Res 2013; 41:487-97; PMID:23104381; http://dx.doi.org/10.1093/nar/gks942.

16. Hopkins JF, Panja S, Woodson SA. Rapid binding and release of Hfq from ternary complexes during RNA annealing. Nucleic Acids Res 2011; 39:5193-202; PMID:21378124; http://dx.doi.org/10.1093/nar/gkr062.

17. Hwang W, Arluison V, Hohng S. Dynamic competition of DsrA and rpoS fragments for the proximal binding site of Hfq as a means for efficient annealing. Nucleic Acids Res 2011; 39:5131-9; PMID:21357187; http://dx.doi.org/10.1093/nar/gkr075.

18. Chao Y, Vogel J. The role of Hfq in bacterial pathogens. Curr Opin Microbiol 2010; 13:24-33; PMID:20080057; http://dx.doi.org/10.1016/j.mib.2010.01.001.

19. Valentin-Hansen P, Eriksen M, Udesen C. The bacterial Sm-like protein Hfq: a key player in RNA transactions. Mol Microbiol 2004; 51:1525-33; PMID:15009882; http://dx.doi.org/10.1111/j.1365-2958.2003.03935.x.

20. Berghoff BA, Glaeser J, Sharma CM, Zobawa M, Lottspeich F, Vogel J, et al. Contribution of Hfq to photooxidative stress resistance and global regulation in Rhodobacter sphaeroides. Mol Microbiol 2011; 80:1479-95; PMID:21535243; http://dx.doi.org/10.1111/j.1365-2958.2011.07658.x.

21. Ding Y, Davis BM, Waldor MK. Hfq is essential for Vibrio cholerae virulence and downregulates sigma expression. Mol Microbiol 2004; 53:345-54; PMID:15225327; http://dx.doi.org/10.1111/j.1365-2958.2004.04142.x.

22. Guisbert E, Rhodius VA, Ahuja N, Witkin E, Gross CA. Hfq modulates the sigmaE-mediated envelope stress response and the sigma32-mediated cytoplasmic stress response in Escherichia coli. J Bacteriol 2007; 189:1963-73; PMID:17158661; http://dx.doi.org/10.1128/JB.01243-06.

23. Sonnleitner E, Schuster M, Sorger-Domenigg T, Greenberg EP, Bläsi U. Hfq-dependent alterations of the transcriptome profile and effects on quorum sensing in Pseudomonas aeruginosa. Mol Microbiol 2006; 59:1542-58; PMID:16468994; http://dx.doi.org/10.1111/j.1365-2958.2006.05032.x.

24. Sittka A, Lucchini S, Papenfort K, Sharma CM, Rolle K, Binnewies TT, et al. Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. PLoS Genet 2008; 4:e1000163; PMID:18725932; http://dx.doi.org/10.1371/journal.pgen.1000163.

25. Ikeda Y, Yagi M, Morita T, Aiba H. Hfq binding at RhlB-recognition region of RNase E is crucial for the rapid degradation of target mRNAs mediated by sRNAs in Escherichia coli. Mol Microbiol 2011; 79:419-32; PMID:21219461; http://dx.doi.org/10.1111/j.1365-2958.2010.07454.x.

26. Sukhodolets MV, Garges S. Interaction of Escherichia coli RNA polymerase with the ribosomal protein S1 and the Sm-like ATPase Hfq. Biochemistry 2003; 42:8022-34; PMID:12834354; http://dx.doi.org/10.1021/bi020638i.

27. De Lay N, Gottesman S. Role of polynucleotide phosphorylase in sRNA function in Escherichia coli. RNA 2011; 17:1172-89; PMID:21527671; http://dx.doi.org/10.1261/rna.2531211.

28. Mohanty BK, Maples VF, Kushner SR. The Sm-like protein Hfq regulates polyadenylation dependent mRNA decay in Escherichia coli. Mol Microbiol 2004; 54:905-20; PMID:15522076; http://dx.doi.org/10.1111/j.1365-2958.2004.04337.x.

29. Rabhi M, Espéli O, Schwartz A, Cayrol B, Rahmouni AR, Arluison V, et al. The Sm-like RNA chaperone Hfq mediates transcription antitermination at Rho-dependent terminators. EMBO J 2011; 30:2805-16; PMID:21673658; http://dx.doi.org/10.1038/emboj.2011.192.

30. Papenfort K, Pfeiffer V, Mika F, Lucchini S, Hinton JC, Vogel J. SigmaE-dependent small RNAs of Salmonella respond to membrane stress by accelerating global omp mRNA decay. Mol Microbiol 2006; 62:1674-88; PMID:17427289; http://dx.doi.org/10.1111/j.1365-2958.2006.05524.x.

31. Massé E, Escorcia FE, Gottesman S. Coupled degradation of a small regulatory RNA and its mRNA targets in Escherichia coli. Genes Dev 2003; 17:2374-83; PMID:12975324; http://dx.doi.org/10.1101/gad.1127103.

32. Horstmann N, Orans J, Valentin-Hansen P, Shelburne SA 3rd, Brennan RG. Structural mechanism of Staphylococcus aureus Hfq binding to an RNA A-tract. Nucleic Acids Res 2012; 40:11023-35; PMID:22965117; http://dx.doi.org/10.1093/nar/gks809.

33. Nielsen JS, Bøggild A, Andersen CB, Nielsen G, Boysen A, Brodersen DE, et al. An Hfq-like protein in archaea: crystal structure and functional characterization of the Sm protein from Methanococcus jannaschii. RNA 2007; 13:2213-23; PMID:17959927; http://dx.doi.org/10.1261/rna.689007.

34. Sauer E, Weichenrieder O. Structural basis for RNA 3'-end recognition by Hfq. Proc Natl Acad Sci USA 2011; 108:13065-70; PMID:21737752; http://dx.doi.org/10.1073/pnas.1103420108.

35. Someya T, Baba S, Fujimoto M, Kawai G, Kumasaka T, Nakamura K. Crystal structure of Hfq from Bacillus subtilis in complex with SELEX-derived RNA aptamer: insight into RNA-binding properties of bacterial Hfq. Nucleic Acids Res 2012; 40:1856-67; PMID:22053080; http://dx.doi.org/10.1093/nar/gkr892.

36. Otaka H, Ishikawa H, Morita T, Aiba H. PolyU tail of rho-independent terminator of bacterial small RNAs is essential for Hfq action. Proc Natl Acad Sci USA 2011; 108:13059-64; PMID:21788484; http://dx.doi.org/10.1073/pnas.1107050108.

37. Ishikawa H, Otaka H, Maki K, Morita T, Aiba H. 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 2012; 18:1062-74; PMID:22454537; http://dx.doi.org/10.1261/rna.031575.111.

38. Olejniczak M. Despite similar binding to the Hfq protein regulatory RNAs widely differ in their competition performance. Biochemistry 2011; 50:4427-40; PMID:21510661; http://dx.doi.org/10.1021/bi102043f.

39. Soper TJ, Woodson SA. The rpoS mRNA leader recruits Hfq to facilitate annealing with DsrA sRNA. RNA 2008; 14:1907-17; PMID:18658123; http://dx.doi.org/10.1261/rna.1110608.

40. Mikulecky PJ, Kaw MK, Brescia CC, Takach JC, Sledjeski DD, Feig AL. Escherichia coli Hfq has distinct interaction surfaces for DsrA, rpoS and poly(A) RNAs. Nat Struct Mol Biol 2004; 11:1206-14; PMID:15531892; http://dx.doi.org/10.1038/nsmb858.

41. Salim NN, Faner MA, Philip JA, Feig AL. Requirement 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 2012; 40:8021-32; PMID:22661574; http://dx.doi.org/10.1093/nar/gks392.

42. Fender A, Elf J, Hampel K, Zimmermann B, Wagner EGH. RNAs actively cycle on the Sm-like protein Hfq. Genes Dev 2010; 24:2621-6; PMID:21123649; http://dx.doi.org/10.1101/gad.591310.

43. Salim NN, Feig AL. An upstream Hfq binding site in the fhlA mRNA leader region facilitates the OxyS-fhlA interaction. PLoS One 2010; 5:5; PMID:20927406; http://dx.doi.org/10.1371/journal.pone.0013028.

44. Wang W, Wang L, Zou Y, Zhang J, Gong Q, Wu J, et al. Cooperation of Escherichia coli Hfq hexamers in DsrA binding. Genes Dev 2011; 25:2106-17; PMID:21979921; http://dx.doi.org/10.1101/gad.16746011.

45. Sauer E, Schmidt S, Weichenrieder O. Small RNA binding to the lateral surface of Hfq hexamers and structural rearrangements upon mRNA target recognition. Proc Natl Acad Sci USA 2012; 109:9396-401; PMID:22645344; http://dx.doi.org/10.1073/pnas.1202521109.

46. Vecerek B, Rajkowitsch L, Sonnleitner E, Schroeder R, Bläsi U. The C-terminal domain of Escherichia coli Hfq is required for regulation. Nucleic Acids Res 2008; 36:133-43; PMID:18000007; http://dx.doi.org/10.1093/nar/gkm985.

47. Sonnleitner E, Napetschnig J, Afonyushkin T, Ecker K, Vecerek B, Moll I, et al. Functional effects of variants of the RNA chaperone Hfq. Biochem Biophys Res Commun 2004; 323:1017-23; PMID:15381101; http://dx.doi.org/10.1016/j.bbrc.2004.08.190.

48. Olsen AS, Møller-Jensen J, Brennan RG, Valentin-Hansen P. C-terminally truncated derivatives of Escherichia coli Hfq are proficient in riboregulation. J Mol Biol 2010; 404:173-82; PMID:20888338; http://dx.doi.org/10.1016/j.jmb.2010.09.038.

49. Lorenz C, Gesell T, Zimmermann B, Schoeberl U, Bilusic I, Rajkowitsch L, et al. Genomic SELEX for Hfq-binding RNAs identifies genomic aptamers predominantly in antisense transcripts. Nucleic Acids Res 2010; 38:3794-808; PMID:20348540; http://dx.doi.org/10.1093/nar/gkq032.

50. Zhang A, Wassarman KM, Rosenow C, Tjaden BC, Storz G, Gottesman S. Global analysis of small RNA and mRNA targets of Hfq. Mol Microbiol 2003; 50:1111-24; PMID:14622403; http://dx.doi.org/10.1046/j.1365-2958.2003.03734.x.

51. Chao Y, Papenfort K, Reinhardt R, Sharma CM, Vogel J. An atlas of Hfq-bound transcripts reveals 3' UTRs as a genomic reservoir of regulatory small RNAs. EMBO J 2012; 31:4005-19; PMID:22922465; http://dx.doi.org/10.1038/emboj.2012.229.

52. Sonnleitner E, Sorger-Domenigg T, Madej MJ, Findeiss S, Hackermüller J, Hüttenhofer A, et al. Detection of small RNAs in Pseudomonas aeruginosa by RNomics and structure-based bioinformatic tools. Microbiology 2008; 154:3175-87; PMID:18832323; http://dx.doi.org/10.1099/mic.0.2008/019703-0.

53. Moon K, Gottesman S. Competition among Hfq-binding small RNAs in Escherichia coli. Mol Microbiol 2011; 82:1545-62; PMID:22040174; http://dx.doi.org/10.1111/j.1365-2958.2011.07907.x.

54. Udekwu KI, Darfeuille F, Vogel J, Reimegård J, Holmqvist E, Wagner EGH. Hfq-dependent regulation of OmpA synthesis is mediated by an antisense RNA. Genes Dev 2005; 19:2355-66; PMID:16204185; http://dx.doi.org/10.1101/gad.354405.

55. Folichon M, Arluison V, Pellegrini O, Huntzinger E, Régnier P, Hajnsdorf E. The poly(A) binding protein Hfq protects RNA from RNase E and exoribonucleolytic degradation. Nucleic Acids Res 2003; 31:7302-10; PMID:14654705; http://dx.doi.org/10.1093/nar/gkg915.

56. Moll I, Afonyushkin T, Vytvytska O, Kaberdin VR, Bläsi U. Coincident Hfq binding and RNase E cleavage sites on mRNA and small regulatory RNAs. RNA 2003; 9:1308-14; PMID:14561880; http://dx.doi.org/10.1261/rna.5850703.

57. Zhang A, Wassarman KM, Ortega J, Steven AC, Storz G. The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. Mol Cell 2002; 9:11-22; PMID:11804582; http://dx.doi.org/10.1016/S1097-2765(01)00437-3.

58. Soper T, Mandin P, Majdalani N, Gottesman S, Woodson SA. Positive regulation by small RNAs and the role of Hfq. Proc Natl Acad Sci USA 2010; 107:9602-7; PMID:20457943; http://dx.doi.org/10.1073/pnas.1004435107.

59. Lease RA, Woodson SA. Cycling of the Sm-like protein Hfq on the DsrA small regulatory RNA. J Mol Biol 2004; 344:1211-23; PMID:15561140; http://dx.doi.org/10.1016/j.jmb.2004.10.006.

60. Sun X, Wartell RM. Escherichia coli Hfq binds A18 and DsrA domain II with similar 2:1 Hfq6/RNA stoichiometry using different surface sites. Biochemistry 2006; 45:4875-87; PMID:16605255; http://dx.doi.org/10.1021/bi0523613.

61. Panja S, Woodson SA. Hfq proximity and orientation controls RNA annealing. Nucleic Acids Res 2012; 40:8690-7; PMID:22761405; http://dx.doi.org/10.1093/nar/gks618.

62. Beisel CL, Updegrove TB, Janson BJ, Storz G. Multiple factors dictate target selection by Hfq-binding small RNAs. EMBO J 2012; 31:1961-74; PMID:22388518; http://dx.doi.org/10.1038/emboj.2012.52.

63. Rajkowitsch L, Schroeder R. Dissecting RNA chaperone activity. RNA 2007; 13:2053-60; PMID:17901153; http://dx.doi.org/10.1261/rna.671807.

64. Geissmann TA, Touati D. Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator. EMBO J 2004; 23:396-405; PMID:14739933; http://dx.doi.org/10.1038/sj.emboj.7600058.

65. Ribeiro EdeA Jr., Beich-Frandsen M, Konarev PV, Shang W, Vecerek B, Kontaxis G, et al. Structural flexibility of RNA as molecular basis for Hfq chaperone function. Nucleic Acids Res 2012; 40:8072-84; PMID:22718981; http://dx.doi.org/10.1093/nar/gks510.

66. Moll I, Leitsch D, Steinhauser T, Bläsi U. RNA chaperone activity of the Sm-like Hfq protein. EMBO Rep 2003; 4:284-9; PMID:12634847; http://dx.doi.org/10.1038/sj.embor.embor772.

67. Vincent HA, Henderson CA, Stone CM, Cary PD, Gowers DM, Sobott F, et al. The low-resolution solution structure of Vibrio cholerae Hfq in complex with Qrr1 sRNA. Nucleic Acids Res 2012; 40:8698-710; PMID:22730296; http://dx.doi.org/10.1093/nar/gks582.

68. Holmqvist E, Reimegård J, Sterk M, Grantcharova N, Römling U, Wagner EGH. Two antisense RNAs target the transcriptional regulator CsgD to inhibit curli synthesis. EMBO J 2010; 29:1840-50; PMID:20407422; http://dx.doi.org/10.1038/emboj.2010.73.

69. Updegrove TB, Correia JJ, Chen Y, Terry C, Wartell RM. The stoichiometry of the Escherichia coli Hfq protein bound to RNA. RNA 2011; 17:489-500; PMID:21205841; http://dx.doi.org/10.1261/rna.2452111.

70. Hopkins JF, Panja S, McNeil SA, Woodson SA. Effect of salt and RNA structure on annealing and strand displacement by Hfq. Nucleic Acids Res 2009; 37:6205-13; PMID:19671524; http://dx.doi.org/10.1093/nar/gkp646.

71. Pfeiffer V, Sittka A, Tomer R, Tedin K, Brinkmann V, Vogel J. A small non-coding RNA of the invasion gene island (SPI-1) represses outer membrane protein synthesis from the Salmonella core genome. Mol Microbiol 2007; 66:1174-91; PMID:17971080; http://dx.doi.org/10.1111/j.1365-2958.2007.05991.x.

72. Hussein R, Lim HN. Disruption of small RNA signaling caused by competition for Hfq. Proc Natl Acad Sci USA 2011; 108:1110-5; PMID:21189298; http://dx.doi.org/10.1073/pnas.1010082108.

73. Galluppi GR, Richardson JP. ATP-induced changes in the binding of RNA synthesis termination protein Rho to RNA. J Mol Biol 1980; 138:513-39; PMID:6157829; http://dx.doi.org/10.1016/S0022-2836(80)80016-7.

74. Steinmetz EJ, Platt T. Evidence supporting a tethered tracking model for helicase activity of Escherichia coli Rho factor. Proc Natl Acad Sci USA 1994; 91:1401-5; PMID:7509071; http://dx.doi.org/10.1073/pnas.91.4.1401.

75. Afonyushkin T, Vecerek B, Moll I, Bläsi U, Kaberdin VR. Both RNase E and RNase III control the stability of sodB mRNA upon translational inhibition by the small regulatory RNA RyhB. Nucleic Acids Res 2005; 33:1678-89; PMID:15781494; http://dx.doi.org/10.1093/nar/gki313.

76. Hämmerle H, Beich-Frandsen M, Ve erek B, Rajkowitsch L, Carugo O, Djinovi -Carugo K, et al. Structural and biochemical studies on ATP binding and hydrolysis by the Escherichia coli RNA chaperone Hfq. PLoS One 2012; 7:e50892; PMID:23226421; http://dx.doi.org/10.1371/journal.pone.0050892.

77. Vogel J, Argaman L, Wagner EGH, Altuvia S. The small RNA IstR inhibits synthesis of an SOS-induced toxic peptide. Curr Biol 2004; 14:2271-6; PMID:15620655; http://dx.doi.org/10.1016/j.cub.2004.12.003.

78. Panja S, Woodson SA. Hexamer to monomer equilibrium of E. coli Hfq in solution and its impact on RNA annealing. J Mol Biol 2012; 417:406-12; PMID:22326348; http://dx.doi.org/10.1016/j.jmb.2012.02.009.

79. Carmichael GG, Weber K, Niveleau A, Wahba AJ. The host factor required for RNA phage Qbeta RNA replication in vitro. Intracellular location, quantitation, and purification by polyadenylate-cellulose chromatography. J Biol Chem 1975; 250:3602-12; PMID:165178.

80. Kajitani M, Kato A, Wada A, Inokuchi Y, Ishihama A. Regulation of the Escherichia coli hfq gene encoding the host factor for phage Q beta. J Bacteriol 1994; 176:531-4; PMID:8288550.

81. Ali Azam T, Iwata A, Nishimura A, Ueda S, Ishihama A. Growth phase-dependent variation in protein composition of the Escherichia coli nucleoid. J Bacteriol 1999; 181:6361-70; PMID:10515926.

82. Sittka A, Sharma CM, Rolle K, Vogel J. 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 2009; 6:266-75; PMID:19333007; http://dx.doi.org/10.4161/rna.6.3.8332.

83. Papenfort K, Said N, Welsink T, Lucchini S, Hinton JC, Vogel J. Specific and pleiotropic patterns of mRNA regulation by ArcZ, a conserved, Hfq-dependent small RNA. Mol Microbiol 2009; 74:139-58; PMID:19732340; http://dx.doi.org/10.1111/j.1365-2958.2009.06857.x.

84. Montero Llopis P, Jackson AF, Sliusarenko O, Surovtsev I, Heinritz J, Emonet T, et al. Spatial organization of the flow of genetic information in bacteria. Nature 2010; 466:77-81; PMID:20562858; http://dx.doi.org/10.1038/nature09152.

85. Diestra E, Cayrol B, Arluison V, Risco C. Cellular electron microscopy imaging reveals the localization of the Hfq protein close to the bacterial membrane. PLoS One 2009; 4:e8301; PMID:20011543; http://dx.doi.org/10.1371/journal.pone.0008301.

86. Persson F, Lindén M, Unoson C, Elf J. Extracting intracellular diffusive states and transition rates from single-molecule tracking data. Nat Methods 2013; In press; PMID:23396281; http://dx.doi.org/10.1038/nmeth.2367.

87. Mehta P, Goyal S, Wingreen NS. A quantitative comparison of sRNA-based and protein-based gene regulation. Mol Syst Biol 2008; 4:221; PMID:18854820; http://dx.doi.org/10.1038/msb.2008.58.

88. Levine E, Zhang Z, Kuhlman T, Hwa T. Quantitative characteristics of gene regulation by small RNA. PLoS Biol 2007; 5:e229; PMID:17713988; http://dx.doi.org/10.1371/journal.pbio.0050229.

89. Slagter-Jäger JG. The role of Hfq in antisense RNA-mediated regulation: a comparison between trans- and cis-encoded RNAs. In: PhD thesis, Uppsala university 2003.

90. Wagner EGH, Altuvia S, Romby P. Antisense RNAs in bacteria and their genetic elements. Adv Genet 2002; 46:361-98; PMID:11931231; http://dx.doi.org/10.1016/S0065-2660(02)46013-0.

91. Romby P, Vandenesch F, Wagner EGH. The role of RNAs in the regulation of virulence-gene expression. Curr Opin Microbiol 2006; 9:229-36; PMID:16529986; http://dx.doi.org/10.1016/j.mib.2006.02.005.

92. Hao Y, Zhang ZJ, Erickson DW, Huang M, Huang Y, Li J, et al. Quantifying the sequence-function relation in gene silencing by bacterial small RNAs. Proc Natl Acad Sci USA 2011; 108:12473-8; PMID:21742981; http://dx.doi.org/10.1073/pnas.1100432108.

93. Pandey SP, Minesinger BK, Kumar J, Walker GC. A highly conserved protein of unknown function in Sinorhizobium meliloti affects sRNA regulation similar to Hfq. Nucleic Acids Res 2011; 39:4691-708; PMID:21325267; http://dx.doi.org/10.1093/nar/gkr060.

94. Bandyra KJ, Said N, Pfeiffer V, Górna MW, Vogel J, Luisi BF. The seed region of a small RNA drives the controlled destruction of the target mRNA by the endoribonuclease RNase E. Mol Cell 2012; 47:943-53; PMID:22902561; http://dx.doi.org/10.1016/j.molcel.2012.07.015.