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Hfq and its constellation of RNA

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Abstract

Hfq is an RNA-binding protein that is common to diverse bacterial lineages and has key roles in the control of gene expression. By facilitating the pairing of small RNAs with their target mRNAs, Hfq affects the translation and turnover rates of specific transcripts and contributes to complex post-transcriptional networks. These functions of Hfq can be attributed to its ring-like oligomeric architecture, which presents two non-equivalent binding surfaces that are capable of multiple interactions with RNA molecules. Distant homologues of Hfq occur in archaea and eukaryotes, reflecting an ancient origin for the protein family and hinting at shared functions. In this Review, we describe the salient structural and functional features of Hfq and discuss possible mechanisms by which this protein can promote RNA interactions to catalyse specific and rapid regulatory responses *in vivo*.

Hfq was discovered in *Escherichia coli* nearly half a century ago (BOX 1) and was one of the first recognized representatives of an extensive RNA-binding protein family, the members of which can be found in almost every cellular organism from all three domains of life¹. The meta-zoan homologues of Hfq include the Sm proteins, named after the autoimmune Sm antibodies that recognize them, and the closely related Sm-like (LSm) proteins, which are also found in single-celled eukaryotes and in archaea. The characteristic feature of the collective Hfq–Sm–LSm protein family is a ring-like, multimeric quaternary architecture that supports interactions with partner macromolecules. Both Hfq and the Sm–LSm proteins have general roles as RNA binders that contribute to post-transcriptional regulation. The Sm–LSm proteins include central components of the mRNA-splicing machinery, scaffolds for RNA-decapping assemblies, and protective chaperones of ribosomal RNAs, small nucleolar RNAs and tRNA precursors¹.

Competing interests statement

The authors declare no competing financial interests.

FURTHER INFORMATION

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SUPPLEMENTARY INFORMATION

See online article: [S1](#) (table)

Today, Hfq is perceived primarily as the core component of a global post-transcriptional network, in which it facilitates the short and imperfect base-pairing interactions of regulatory small RNAs (sRNAs) with *trans*-encoded target mRNAs. Model organisms such as *E. coli* or *Salmonella enterica* can express ~100 different sRNAs. Unlike their functional equivalents in eukaryotes — the 22-nucleotide-long microRNAs — these bacterial sRNAs are heterogeneous in size and structure. The intriguing physiological functions of Hfq and sRNAs have recently been reviewed^{2–5}.

There are several general mechanisms of Hfq-mediated regulation at the levels of translation or RNA stability^{2–7}, and these are summarized in FIG. 1. First, Hfq can suppress protein synthesis by aiding a cognate sRNA to bind the 5' region of its target mRNA, thus rendering this 5' region inaccessible for translation initiation (FIG. 1a). Conversely, Hfq can boost translation by guiding an sRNA to the 5' region of its target mRNA in order to disrupt a secondary structure that otherwise inhibits ribosome binding (FIG. 1b). Prior to target recognition, Hfq can protect sRNAs from ribonuclease cleavage (FIG. 1c) or present some RNAs in such a way as to promote mRNA cleavage (FIG. 1d). Finally, Hfq can promote RNA turnover by rendering the 3' ends accessible for polyadenylation and subsequent 3'-to-5' exonucleolytic degradation (FIG. 1e). In each case, the precise mechanism of action of Hfq seems to depend on the structural information encoded in the RNA molecules with which the protein interacts.

Although many aspects of Hfq function have been discovered, fundamental mechanistic features remain unclear or are only just coming to light. For example, until recently few cellular targets of Hfq were known, but it is becoming apparent that the protein interacts dynamically with a plethora of different RNA species and has an evolutionarily conserved preference *in vivo* for sRNA and mRNA partners^{8–13}. There is also a growing recognition of the potentially complex behaviour of sRNAs themselves: these RNAs were previously thought to have single-target specificity, but increasing numbers have been shown to act on multiple mRNAs; likewise, more mRNAs are emerging as shared targets of multiple cognate sRNAs^{14,15}. Hfq-dependent regulators now include sRNA families with several homologues^{16–18}, hierarchically acting sRNAs^{19,20}, RNA decoys^{21,22} and transcripts with dual sRNA and mRNA function^{23,24}. Given the many cellular RNA partners of Hfq, protein availability becomes an important mechanistic consideration; although the protein was always assumed to be sufficiently abundant, it has been shown that sRNAs and mRNAs compete for Hfq binding within the cell^{25–27}. How can one small protein facilitate so many diverse RNA encounters in an interwoven regulatory network that is beginning to rival in complexity the intricate fabric of transcriptional regulation? Here, we discuss the structural and mechanistic features that endow Hfq with the capacity to serve its complex biological functions as a facilitator of regulatory sRNA–mRNA interactions.

Structural basis for RNA binding by Hfq

The characteristic ring architecture of the Hfq–Sm–LSm family is generated either from identical protomers, as found in the hexameric Hfq (FIG. 2), or from non-identical protomers to form hetero-oligomers, as found in the heptameric Sm core of the human spliceosome. High-resolution crystal structures of Hfq, Sm and LSm proteins from

numerous bacteria and archaea (Supplementary information S1 (table)) reveal that the structure of the protomers and their quaternary organization are well conserved, despite the great divergence of their primary sequences. The conserved core of the Hfq protomer, which consists of an α - β_{1-5} fold, comprises ~65 residues and, in most cases, is the only part that has been structurally resolved (Supplementary information S1 (table)). As it stands, both the structure and function of the variable carboxyl termini of Hfq proteins are little understood (BOX 2).

The conserved core harbours two sequence motifs named Sm1 and Sm2 (32 and 14 amino acids long, respectively) that form conserved secondary-structural elements²⁸ (FIG. 2a). These motifs are a distinctive signature for the Hfq-Sm-LSm family and are likely to have originated from an ancient ancestral protein. The Sm1 motif encompasses the first three strands (β_{1-3}) of the common α - β_{1-5} structural core (FIG. 2b) and is highly conserved throughout the family^{29,30}. Motif Sm2 is formed by the last two strands (β_{4-5}) and differs between the bacterial Hfq proteins on the one hand and the archaeal and eukaryotic Sm and LSm proteins on the other. As the Sm2 elements occupy the protomer interfaces (FIG. 2c), this motif may influence the number of subunits in the ring and the specificity of their interactions to form the hetero-oligomers that are observed for the Sm proteins. Hetero-oligomers may also occur in bacteria that express multiple Hfq homologues³¹. In addition, Hfq variants with two sets of Sm domains seem to exist and may be expressed as naturally linked dimers³².

The ring-like architecture of Hfq exposes two faces for potential interaction with nucleic acids. To distinguish them, we use the common definition of the ‘proximal face’ as the surface on which the amino-terminal α -helix is exposed and of the ‘distal face’ as the opposite side (FIG. 2d). As outlined further below, the faces interact differently with RNA, resulting in intriguing sequence preferences.

Two RNA-binding faces

RNA threads through the central pore of the hetero-heptameric Sm ring in the U1 small nuclear ribonucleoprotein (snRNP) of the human spliceosome³³. By contrast, Hfq has a much smaller central pore and binds the RNA in circular grooves on either face of the ring in the available co-crystal structures (FIG. 2d and Supplementary information S1 (table)). In the complex formed by *Staphylococcus aureus* Hfq and the U-rich heptamer AUUUUUG, the RNA forms a small ring in a recessed groove on the proximal face, in which each protomer contacts a single nucleotide³⁴. The RNA is contacted through amino acid side chains in the inter-strand loops, and the base-pairing edges of the nucleotides are exposed to the solvent (although not in a suitable orientation for antisense pairing with a potential partner).

How the Hfq distal face interacts with RNA has been illuminated by the crystal structure of the *E. coli* protein in complex with poly(A) RNA³⁵. In this complex, each protomer contacts three bases, in contrast with the single-base contacts on the proximal face. The base-pairing edges of two nucleotides of the triplet are sequestered in an intra-protomer groove. The third base is exposed to the solvent in an orientation that is potentially compatible with pairing and stacking against bases from another RNA, but we are not aware of any evidence for

such an interaction. The distal face seems to prefer the sequence motif ARN or ARNN (in which R is a purine and N is any base)³⁵. A similar motif, AAYAAYAA (in which Y is a pyrimidine), was enriched in genomic selection experiments for Hfq-associated RNA³⁶, indicating that the RNA species containing this motif may have bound principally to the distal face.

The distinct sequence preferences for the proximal and distal faces that are implied by the crystal structures (that is, single-stranded, U-rich sequences for the proximal side and single-stranded ARN or ARNN motifs for the distal side) are consistent with the results of a systematic mutagenesis of *E. coli* Hfq; this analysis identified face-selective binding of natural RNA molecules and established a model of how Hfq can simultaneously bind an sRNA and its target mRNA³⁷. However, the two Hfq surfaces may not be exclusively an 'sRNA face' and an 'mRNA face', and some RNAs may bind to both faces³⁸. Nonetheless, having distinct binding faces promotes the recruitment of multiple RNAs on one Hfq molecule. The repetitive nature of each surface enables contact at several sites with the same RNA to give rise to cooperative effects³⁹, and facilitates the recruitment of competitor RNAs to favour exchange with stably bound RNA³⁸.

Recognition of different RNA species

Diverse RNA species co-immunoprecipitate with Hfq, but the greatest enrichment is typically for sRNAs and mRNAs^{9–11}, despite the tremendous excess of potential competitor cellular RNAs such as tRNAs and rRNAs. Even remote Hfq homologues (from the bacteria *Neisseria meningitidis* and *Aquifex aeolicus*, and the archaeon *Methanocaldococcus jannaschii*) preferentially interact with sRNAs when expressed heterologously in *S. enterica*¹¹. So, what are the determinants of sRNAs and mRNAs that are preferentially recognized by Hfq?

Hfq-associated sRNAs are diverse in length (50–250 nucleotides) and, although commonly devoid of ORFs, they still exceed the size of the regions in which they would be accommodated on the proximal and distal faces of Hfq, according to the crystal structures of Hfq–RNA complexes. These sRNAs seem to fold into stem–loops and are likely to bear modular domains for sRNA biogenesis or function (FIG. 3). Highly conserved regions within homologous sRNAs occur at the 5' end or internally and typically serve as target recognition domains that select the regulated mRNAs by short pairing^{40–46}; we refer to these as 'seed' regions, in loose analogy with the cognate elements of eukaryotic micro-RNAs. The common stem–loop structure at the 3' end of sRNAs, followed by a short poly(U) stretch, is part of a Rho-independent transcription terminator and probably serves the additional function of preventing attack by 3' exonucleases (provided that it is not too long). In several cases, Hfq binds in an AU-rich single-stranded region upstream of the terminator^{25,47–50}, and this binding might then expose the target-binding domain of sRNAs for the interrogation of potential mRNA partners. However, as most sRNAs are short and structured, the proximity of the stem–loop might be coincidental. In contrast with the mRNA-binding 'seed' domain, the potential contact sites of Hfq in sRNAs seem to have weak conservation at the nucleotide level and therefore remain elusive to prediction.

A representative Hfq-binding site in an mRNA is the repetitive sequence AANAANAANAAN, found in the 5' untranslated region (UTR) of the *rpoS* transcript (encoding RNA polymerase σ -factor S) in *E. coli*. This element matches well with the aforementioned ARN motif for binding to the distal face of Hfq (as deduced from the crystallographic data³⁵), and it is critically required by the various sRNAs that activate *rpoS* translation⁵¹. Regulation mediated by this site seems to depend on context, as another high-affinity Hfq site nearby, an AAAAAA motif, cannot support an sRNA response⁵¹.

Pattern searches in the 5' UTRs of *E. coli* mRNAs indicate the frequent occurrence of ARN motifs, especially overlapping the ribosome-binding site (RBS), the region to which many sRNAs bind to suppress translation initiation³⁵. The Hfq aptamer sequence AAYAAAYAA is enriched around the start and stop codons of mRNAs³⁶, consistent with the high recovery of UTRs by deep sequencing of Hfq-bound RNA *in vivo*¹⁰. However, all the global approaches undertaken thus far have considerable bias, and on balance the accumulating *in vitro* Hfq-binding data for individual mRNAs do not necessarily support a uniform, single-stranded binding motif⁵². Thus, for faithful predictions of where Hfq binds to mRNAs, global mapping of *in vivo* binding sites by experiment seems necessary.

The available structural data show how Hfq interacts with single-stranded RNA, but it is likely that the protein will also engage folded RNA. Recognition of RNA folds might explain the reported binding of Hfq to tRNA (both mature and precursor forms) and rRNA⁹. Evidence indicates that Hfq associates with the short T-stem and D-stem structures in tRNA^{53,54}, as well as with an sRNA with an elaborate pseudoknot fold⁵⁵. However, not all structured RNAs are readily bound by Hfq⁵⁶, and a general code for Hfq binding with predictive power for individual RNA molecules is yet to be determined, if such a thing even exists. From an evolutionary perspective, a stringent binding motif might not be beneficial, as it may restrict potential sampling of existing cellular RNAs by newly acquired sRNAs or targets.

The role of RNA termini

The life history of a cellular RNA is reflected by the chemical moieties at its termini; for example, nascent RNAs have a 5'-triphosphate and a 3'-hydroxyl group, whereas the presence of a 5'-hydroxyl group or a 5'-monophosphate marks a processed RNA. Whether the chemical status of the sRNA termini affects their activity or recruitment to Hfq remains to be explored. Most sRNAs accumulate as the primary transcript with a 5'-triphosphate group. However, some undergo endonucleolytic processing to accumulate as a shorter 3' form^{8,57,58} with a newly generated, conserved 5' end for target interactions^{26,51,59}, and other Hfq-bound sRNAs are cleaved from polycistronic mRNAs^{21,22,60}. In both situations, ribonuclease E (RNase E), an endoribonuclease, is the likely cleaving factor, and it generates a 5'-monophosphate on these processed sRNAs. If these termini affect Hfq binding, then processing of sRNAs could regulate their functions. Note that there are also cases for which processing leads to accumulation of a 5' form of an sRNA^{19,61}, but if cleavage is mediated by RNase E, this will generate a 3'-hydroxyl group, as in the primary transcript.

Several sRNAs have been reported to have poly(A) tails^{19,57,60}. Hfq may associate with poly(A) polymerase, either directly or indirectly, and this association may contribute to the addition of poly(A) tails on certain sRNAs^{62–64}. Polyadenylation of an sRNA might alter its binding properties, for example, to hypothetically switch the preferred binding site on Hfq from the proximal to the distal face.

As mentioned above, another type of tail that is found on most sRNAs is a stretch of 6–8 uridines that results from Rho-independent termination. Accumulating evidence indicates that these short poly(U) tails could be one of many redundant recognition determinants that influence the interactions of the sRNA with the proximal face of Hfq^{65,66}.

Main pathways of Hfq–sRNA action

Now we can turn to the question of how exactly Hfq facilitates sRNA-mediated regulation in the cell. On the basis of *in vitro* data^{48,50,67–70}, it is generally assumed that the crucial encounter between an sRNA and its cognate mRNA leads to a productive tripartite Hfq–sRNA–mRNA complex. But is this complex really the biologically active entity? In fact, the available data support a scenario in which the paired sRNA–mRNA complex, released from Hfq, acts as the key regulatory determinant (FIG. 4a). Most sRNA-binding sites in transcripts are well positioned for successful competition with ribosome binding, and numerous studies have shown that *in vitro*-synthesized sRNAs or even shortened versions can suppress translation of cognate mRNAs by either directly masking the RBS^{42,71–73}, or sequestering other sensitive sites in the 5' UTR^{18,43,70} or proximal coding sequence⁷⁴. Hfq-mediated annealing of sRNAs to cognate mRNAs has been amply demonstrated *in vitro*^{50,67}. Importantly, after the RNA duplexes have formed *in vitro*, they remain stable even if Hfq is removed (or disintegrated proteolytically)^{41,67,75}, fulfilling the criterion for Hfq being a catalyst of inter molecular RNA pairing. Likewise, translation activation by Hfq-associated sRNAs works *in vitro*, and it is the sRNA–mRNA duplex and not Hfq that is essential for translation activation^{19,51}. One can imagine that after an sRNA has disrupted an inhibitory structure around the RBS, initiating ribosomes will maintain the open state of a target by preventing its refolding.

Accordingly, the tripartite Hfq–sRNA–mRNA complex might be viewed as a 'transition state', with Hfq as the catalyst (FIG. 4a). Is it possible, in some cases, for sRNA to be the catalyst, to generate yet another type of functional active complex? For example, the sRNA could leave and Hfq could remain on the mRNA as the executor of function. Although Hfq by itself can repress translation *in vitro*^{19,76,77}, there has not been experimental evidence for this being the result of sRNA-mediated regulation *in vivo*. Finally, we note that the tripartite complex may be sufficiently stable to recruit additional components, such as RNase E, a scenario to which we return below.

Role of Hfq in RNA duplex formation

The role of Hfq in RNA duplex formation is probably a key aspect of its contribution to regulatory processes. Early during the encounter stage, Hfq may behave as a catalyst of inter-molecular RNA pairing (FIG. 4b). Like a conventional enzyme, Hfq could catalyse the process by reducing the entropy penalty for encounters between the reactants — for

example, by simply tethering one or more RNAs to bring them into proximity, so that their pairing is favoured. Also in analogy with a conventional enzymatic mechanism, Hfq could drive reactions through ‘induced fit’: if binding of one RNA to Hfq influences the protein structure to favourably present base-pairing regions of the RNA, then Hfq may catalyse pairing by surmounting kinetic folding traps.

Thus, there are several possible routes for Hfq to aid sRNA–mRNA duplex formation. First, Hfq could increase the on rate for sRNA annealing to a target^{18,38,41,50,67,78–80}. Fast initial RNA contacts are the hallmark of classical bacterial *cis*-antisense systems⁸¹, and such contacts must also be crucial for Hfq-dependent regulators: in the absence of Hfq, the sRNA–mRNA pairs usually exhibit *in vitro* formation rates^{41,79} that are far too slow to be compatible with a biologically relevant time frame *in vivo*^{40,68,82}.

Second, Hfq could favour the release of sRNA–mRNA as a stable pair. Indeed, the cognate sRNA–mRNA duplexes facilitated by Hfq are more stable than those formed without the protein. This increased duplex stability could also help an sRNA to outcompete intramolecular structures within its target mRNA (especially in the case of target activation). Work on the *rpoS* mRNA and its multiple cognate sRNAs concluded that stabilization of the sRNA–mRNA complexes, rather than an altered on rate, is the underlying cause of *in vivo* regulation⁵¹.

Third, Hfq could (and has been shown to) induce changes in RNA structure to favour duplex formation^{48,67}. The potential need of Hfq-mediated remodelling in some systems is highlighted by observations that shorter fragments of some sRNAs are better translational repressors than the full-length sRNAs, at least *in vitro*^{74,83}. However, whether such antecedent remodelling by Hfq is physiologically important is yet to be verified *in vivo* with suitable mutations in the sRNA genes.

Intracellular kinetics and competition for Hfq

For an sRNA to exert its regulatory activity on a target — and, conversely, for a target mRNA to be regulated by an sRNA — both partners must gain access to Hfq. However, there is potentially a tremendous obstacle to the ternary encounter, in the form of the overwhelming excess of cellular RNAs that might sequester and exhaust the pool of available Hfq. This impasse could be circumvented if Hfq could rapidly associate with and quickly release RNA molecules (either alone or in complex with a matching partner). However, the *in vitro* half-lives of Hfq–RNA complexes (>100 min) are too long to be compatible with the 1–2 min response times that are seen *in vivo*^{40,68,82}.

One solution to this conundrum is for Hfq to be continuously released from the sRNA–mRNA complex as a result of RNase E-mediated, coupled degradation of the two RNAs *in vivo*⁸⁴. A more general solution is a dynamic RNA-cycling model³⁸ in which Hfq-bound RNA molecules are continuously displaced by the many cellular competitor RNAs (FIG. 5). In this model, RNA molecules initially bind to one of the Hfq subunits but then might chase each other over the surface of the hexameric ring until they are dislodged by another free RNA awaiting an opportunity to associate. This model can account for observations that the *in vitro* Hfq-dissociation rates of many sRNAs and their targets are primarily determined by

the concentration of competitor RNAs^{78,80,85}. One can easily see how the structure of Hfq, with six equivalent binding sites and two surfaces, is well constructed for this rapid RNA cycling, because multiple RNA molecules can be loosely bound in parallel and jostle among each other for further interactions.

A little accounting

Considering that Hfq must facilitate many different RNA–RNA encounters and perhaps also higher-order assemblies with ribonucleases or other proteins, the location and precise concentration of Hfq molecules become key operating parameters. Unfortunately, despite a supposed abundance of Hfq, experimental measurements of its copy number have been reported only for *E. coli*, and then with an enormous discrepancy in the observed values, which range from ~400 hexamers per cell⁸⁶ to ~10,000 hexamers per cell^{87,88}. The available data indicate that Hfq synthesis is autoregulated at the translational level⁸⁹, and this autoregulation is expected to result in a constant intracellular concentration during rapid growth phase. However, as cells move to stationary phase, Hfq copy number decreases threefold^{87,88}.

Although the cellular concentrations of Hfq ligands are largely unknown, there is likely to be a perpetual excess of potential RNA partners, given the dozens of sRNAs with copy numbers in the range of 10 to 10³ molecules per cell^{26,90,91}, the hundreds of mRNAs that co-purify with Hfq^{9,10}, and the abundant stable RNAs^{53,54}. Despite the uncertainties about the exact number of cellular Hfq molecules, the range corresponds to micro-molar concentrations; together with the nanomolar dissociation constants of Hfq–RNA interactions³⁸ and the excess of RNA ligands, this concentration range predicts that the RNA-binding sites of Hfq are always fully occupied *in vivo*. However, most bacterial mRNAs are present in low numbers⁹², and many sRNAs have brief lifetimes^{60,84}. Thus, if Hfq is fully saturated, there are limited opportunities for an sRNA and its target mRNA to encounter each other on Hfq, let alone to elicit the rapid regulatory responses observed, which are in the order of 1 min^{68,84}. So how are these obstacles overcome?

The aforementioned RNA-cycling model³⁸ might help to explain how an sRNA overcomes the difficulty of loading onto an already saturated Hfq to achieve a fast regulatory response: this will happen if there is a local increase in sRNA concentration that might overwhelm the Hfq-bound RNA. However, it is currently unclear how a high local concentration could be maintained, especially as a typical sRNA is predicted to diffuse the length of the *E. coli* cell in a fraction of a second. A related logistical difficulty of loading a busy Hfq with new sRNAs is that any sudden increase in one particular sRNA (for example, as a result of oxidative stress⁹¹) may affect many unrelated mRNAs if Hfq is a limiting factor, so that there is effectively a short-circuiting of other Hfq-mediated regulatory networks²⁷.

Evidence for short-circuiting of Hfq networks was originally sought from the negative effects of highly expressed sRNAs on mRNA translation⁹¹ or Hfq-bound RNA²⁶. Depletion of the available Hfq is predicted to occur when sRNAs and target mRNAs are transcribed at high levels without their partners. However, when the transcription of both species is synchronized such that they are expressed concomitantly, the sequestration of Hfq is minimized²⁷. This observation suggests that the availability of Hfq is indeed limiting, and

that the activities of the different networks that are mediated by sRNAs are therefore highly intermeshed. Perhaps one way of creating a local concentration burst of an sRNA while avoiding short-circuiting effects is through compartmentalization such that the sRNA is synthesized near the targeted transcripts. This would require a three-dimensional organization of genes in chromatin, and there is some evidence for clustering of transcriptional units⁹³. Whether it is such compartmentalization or another process that explains the puzzle of sRNA loading *in vivo* awaits insights from further experimental investigations.

Protein interactions and functional partners

Like its Sm–LSm homologues, Hfq may recruit protein partners *in vivo*. For example, the LSm protein (also known as Snp) of the archaeon *Haloferax volcanii* associates with translation elongation factors EF2 and EF1 α 1 as well as with tRNA synthetases⁹⁴. Similarly, Hfq has been copurified with other proteins that act on RNA, such as polynucleotide phosphorylase (PNPase), in complex with poly(A) polymerase (PAP) from *E. coli*⁶⁴. Intriguingly, the exoribonuclease PNPase has also been identified as a major functional partner for Hfq in mediating sRNA turnover and activity⁹⁵.

Comprehensive studies of protein–protein interactions in *E. coli*^{96,97} have predicted functional if not physical associations of Hfq with multiprotein complexes, including RNA polymerase, the ribosome and the RNase E-based degradosome. These large-scale network predictions ultimately require experimental validation, and there is already evidence that some of these predicted interactions of Hfq (for example, with cold-shock-like protein CspC) are indirect and occur via coincidental binding to the same RNA molecule⁹⁸. Interestingly, RNA may influence the interaction of Hfq with a recently identified partner, the transcription termination factor Rho in *E. coli*, to promote anti-termination⁹⁹. Thus, the modulation of transcription termination by Hfq may be another of its RNA-mediated regulatory activities.

There may be other important protein partners of Hfq *in vivo*, some of which may form transient complexes that are difficult to detect by conventional approaches. The data that are currently available indicate that the prime candidate for a direct partner of Hfq is the endoribonuclease RNase E, to which we now turn.

Hfq and RNase E

RNase E, the dominant catalyst of general mRNA turnover in gammaproteobacteria¹⁰⁰, is the main factor in sRNA-induced decay of target mRNAs^{44,61,78,84,101–104}. RNase E also degrades the sRNAs, both when they are unpaired^{105–107} and when they are paired with targets; the latter process is referred to as coupled degradation⁸⁴.

Intriguingly, immunoprecipitation experiments have predicted an Hfq–RNase E protein complex that co exists, perhaps in a mutually exclusive manner, with the larger RNase E-based degradosome^{102,108}. The proposed interaction requires the same region of RNase E that otherwise binds the degradosome-specific ATP-dependent RNA helicase RhlB¹⁰⁹, and is supported by the observation that the deletion of this region eliminates co-purification of

Hfq with RNase E¹⁰⁹ and negatively affects mRNA destruction by several sRNAs^{44,69,84,102,103,108,110}. Two sRNAs were found to be enriched by co-immunoprecipitation with RNase E, supporting a model of a ribonucleoprotein complex in which Hfq, guided by an sRNA, increases the local concentration of RNase E for programmed mRNA destruction¹⁰², either proximal^{78,103} or distal¹¹⁰ to the site of base-pairing.

Whether Hfq needs to leave an RNA duplex before RNase E acts has not been investigated. A related important question is how Hfq-bound RNase E would be prevented from indiscriminately cleaving any mRNA that happens to be in the vicinity. One recent study suggested a licensing effect, such that mRNA cleavage (at a distal site) proceeds only when an sRNA unmask the mRNA of protective ribosomes, and RNase E is simultaneously localized to the 5' region of the mRNA, where the nuclease works best¹¹⁰. A similar effect could occur in cases for which the binding of sRNA induces a structural change in the target to unveil a cryptic cleavage site of RNase E⁷⁸. Another possibility is that the recruitment of Hfq to RNase E requires an unknown RNA intermediary, a hypothesis that is supported by studies with purified components¹¹¹.

An attractive model for the determination of cleavage specificity holds that the sRNA–mRNA duplex itself serves as the signal for target cleavage. Although RNase E prefers single-stranded, AU-rich substrates, the enzyme can also be directed to cleave at sites of RNA duplexes and at more complex folds^{112,113}. This mode of recognition might be particularly useful when sRNAs target the coding sequence^{46,103} and have to out-race elongating ribosomes. Obviously, a direct recruitment of RNase E to sRNA–mRNA duplexes could work with or without Hfq, but the complex formation with Hfq might secure sufficiently high levels of RNase E at the site of action.

For many RNAs, RNase E acts at higher rates when the 5'-terminal group of the substrate is a monophosphate^{100,112,113}. Nascent transcripts have a triphosphate cap, and a 5'-monophosphate terminus may be generated by the activity of RNA pyrophosphohydrolase (RppH)¹⁰⁰. This 'decapping' enzyme is another interesting candidate for an Hfq-interacting protein, according to protein network analyses^{96,97}. If this interaction did occur, Hfq might be able to facilitate decapping of a sequestered mRNA and make it a better substrate for RNase E. Furthermore, a decapped sRNA that was associated with Hfq might, in principle, be a more effective guide to target mRNA degradation by RNase E. The postulated role of Hfq as a decapping facilitator would bear functional analogy to the human mRNA decapping–degradation complex, which has an LSM core¹¹⁴. Structural data are required to corroborate these predictions and identify where potential proteins might bind on the Hfq ring.

Subcellular localization of Hfq

Hfq might localize in subcellular pockets, as suggested by various studies that have detected Hfq primarily in the cytoplasm¹¹⁵, at the cytoplasmic membrane¹¹⁶ or in the nucleoid⁸⁷. Localization near the membrane would fit with the observation that an Hfq-dependent sRNA downregulates the mRNA encoding an inner-membrane protein only if the nascent protein is

membrane localized¹¹⁷. The potential localization in the DNA nucleoid is consistent with the finding that Hfq binds duplex DNA with an apparent dissociation constant of ~400 nM¹¹⁸. The DNA-binding properties of Hfq have been little explored, and this topic might warrant deeper investigation given that some aspects of genetic regulation are not explained solely by effects at the RNA level¹¹⁹.

Early studies suggested that Hfq co-localizes with the ribosome and, specifically, with the 30S subunit^{87,120–123}, but a recent study does not support this ribosome association¹²⁴. However, the interactions might be transient and depend on active translation, which would explain the observation that sRNA and target mRNA can both be loaded onto 30S subunits in the early translation pre-initiation complex¹²².

Concluding comments

Within the ever-expanding universe of Hfq–Sm–LSm proteins, the sub-group of the homomorphic Hfq proteins has evolved into a distinctive cluster that functions principally to facilitate the activity of sRNA adaptors in bacterial regulatory networks. This broad capacity distinguishes Hfq from the heteromorphic Sm and LSm proteins of eukaryotes, which may be more specialized in function¹²⁵. The evolutionary tree of Hfq has some interesting off-shoots, and although our attention has long been centred mostly on the branch of Hfq and affiliated sRNAs in Gram-negative *E. coli* and *S. enterica*, considerable variations on this theme are now coming into the limelight, including sRNA-mediated regulation in bacteria with very different or multiple Hfq homologues^{126,127}. Our understanding of how Hfq plays wide-ranging regulatory roles has been tremendously advanced by structural and functional studies; nonetheless, several fundamental aspects remain unclear.

One topic for further exploration is how Hfq, including its flexible tail, engages natural sRNAs and the target transcripts. The Hfq–sRNA–mRNA interaction is effectively a three-body problem, and the outcome is difficult to predict a priori. Indeed, from scrutiny of the available crystal structures and biochemical data, it is clear that any ‘recognition code’ of Hfq for RNAs is likely to be complex and highly context dependent. Deciphering such a code and addressing many of the other questions concerning Hfq functions demand an understanding of the behaviour of Hfq in a cellular context. Accordingly, a global map of Hfq interaction sites *in vivo* obtained by covalent crosslinking combined with RNA deep sequencing¹²⁸ should be illuminating. It should also provide insight into the kinetics of Hfq recruitment, for example, in response to stress and other physiological changes.

For understanding activity rates, it is important to establish whether the cellular concentration of Hfq varies. Related to this question is the unexplored topic of how Hfq assembles and disassembles *in vivo* (that is, whether cellular chaperones aid biogenesis and turnover), and whether this is a regulated process. Of note, a recent genetic screen for factors involved in regulation by Hfq-associated sRNAs in *E. coli* identified many point mutations in the *hfq* gene that resulted in dramatically different *in vivo* copy numbers of the protein⁹⁵.

Another promising topic for detailed study is the regulatory interactions of Hfq with other cellular factors. In particular, structural information on the interactions of Hfq–RNA

complexes with RNase E are anticipated to explain how the RNA might be remodelled as a substrate through both protein–protein and RNA–RNA interactions. Extrapolating from the available data, we envisage a dynamic cellular theatre, in which the staging sites of Hfq are always saturated by nucleic acid players but there is a rapid exchange of partners and a high flux through RNA turnover, as well as the possibility of interactions with modulating protein partners. Perhaps *in vivo* the exchange of RNA on Hfq is assisted by an energy-dependent proof-reading step that improves the accuracy of sRNA-mediated processes, analogous to the mechanisms that ensure translational fidelity¹²⁹. An Hfq-dependent proof-reading mechanism for sRNA–mRNA pairing could help rationalize how specificity is achieved when the recognition ‘seed’ region of the sRNA is small and the base-pairing complementarity with targets is imperfect.

Consideration of the dynamic situation *in vivo* naturally leads to another key question concerning when and where Hfq has the opportunity to interact with RNAs; the extent to which compartmentalization has a role in Hfq meeting sRNA and mRNA partners is presently unclear. Recent results indicate that the intracellular diffusion rates of certain mRNAs are low, suggesting that these particular molecules remain close to their sites of transcription¹³⁰. However, some transcripts, particularly those encoding membrane proteins, may move to other sites for translation¹³¹. Whether mRNAs and sRNAs move far from their sites of synthesis or stay nearby also has important implications for the postulated Hfq–sRNA–RNase E complexes; given their total molecular weight of at least 600 kDa, which will diminish intracellular diffusion¹³², will these complexes still allow an associated sRNA to pursue its target?

Regardless of whether a transcript diffuses little or is actively transported to a specific location, most mRNAs spend most of their lifetimes in polysomes. Therefore, Hfq might gain access to an mRNA at early and late stages of transcription, and at the leading end of the polysome. Recent evidence for interactions of Hfq and RNase E at the 5′ UTR provides a model for how the assembly might be triggered by sRNA to act at a distance within a disassembling polysome¹¹⁰. It is striking that bacterial genomes have a selective bias for AU-rich regions at both 5′ and 3′ UTRs¹³³, which seem to be the preferred sites for Hfq binding. Whether these regions do reflect binding sites for Hfq remains to be established. The precise localization of RNAs and Hfq *in vivo* is a key area in which future efforts are likely to be rewarded with much functional insight.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Chaperones	Protective carriers for a meta-stable state of a macromolecule; for proteins, chaperones assist protein folding, and in the context used here, a chaperone confers protection to RNA species that are vulnerable to chemical or enzymatic attack.
RNA decoys	Cellular RNAs that inactivate regulatory RNAs by mimicry of their actual targets.
Protomers	Subunits of an oligomeric assembly.
Hetero-oligomers	Protein assemblies in which the subunits are not chemically identical.
Spliceosome	The series of multicomponent assemblies that dynamically remodel and cleave introns from eukaryotic mRNAs.
Trans-actions	Regulatory base-pairing of a small RNA with a <i>trans</i> -encoded target mRNA.
Rho-independent transcription terminator	A stable secondary RNA structure followed by a short poly(U) stretch that destabilizes the RNA–DNA duplex during transcription so that the RNA polymerase falls off.
Aptamer	An oligonucleotide that is selected <i>in vitro</i> from a large population of combinatorial variants for a targeted property, such as binding to a defined protein.
Pseudoknot	A structure that is formed when duplex-forming regions are interwoven, so that half of one duplex is intercalated between the two halves of another duplex.
Poly(A) polymerase	An important enzyme that catalyses the addition of adenosine to the 3' end of mRNA and thereby accelerates RNA turnover <i>in vivo</i> .
On rate	In classical reaction kinetics, the rate of complex formation, with dimensions of concentration per unit time for simple binary associations.
Polynucleotide phosphorylase	(PNPase). An exoribonuclease that uses phosphate as an attacking group to sequentially liberate nucleoside 5'-diphosphates from the 3' end of an RNA.
Degradosome	A multi-enzyme assembly that was first identified in <i>Escherichia coli</i> and is found in many other bacterial lineages. In <i>E. coli</i> , the canonical components are ribonuclease E and polynucleotide phosphorylase, as well as a DEAD-box RNA helicase and the glycolytic enzyme enolase.

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Box 1**A brief history of Hfq research**

Research on Hfq commenced in the late 1960s, when the protein was identified in *Escherichia coli* as an essential host factor of the RNA bacteriophage Q β (from which the name Hfq was derived)¹³⁴; the protein probably improves the replication efficiency of the viral genome by melting a secondary structure at the 3' end of the RNA¹³⁵. Early biochemical characterization defined *E. coli* Hfq as a remarkably heat-resistant and abundant nucleic acid-binding protein with strong preferences for AU-rich single-stranded RNA^{86,136–140}.

The 1990s brought the first clues as to the potential benefits that Hfq could provide to the bacterium itself, rather than to its phage predator. Loss of Hfq was found to reduce fitness and impair the stress response, and (in pathogenic bacteria such as *Brucella abortus*) to diminish virulence^{141,142}. In addition, it was discovered that the translation or turnover of numerous cellular mRNAs is regulated by Hfq^{76,143,144}.

About a decade ago, structural and bioinformatic studies showed that Hfq is part of the much wider Sm family, highlighting that its origins date back to the last common ancestor of eukaryotic, bacterial and archaeal lineages^{50,67}. It also became clear that Hfq associates with small regulatory RNAs (sRNAs) to promote their base-pairing with cognate target mRNAs^{8,50,67,145}. The sRNA–mRNA pairing affects the translation rate and lifetime of the targeted transcript. The connection of Hfq as a facilitator of the *trans*-actions of sRNAs could account for many of the complex phenotypic effects that are observed in the early gene knockout studies^{9,141}. Hfq proteins have been predicted to be present in at least 50% of all bacterial species². In addition, unusual functional homologues with weak homology to *E. coli* Hfq are still being discovered^{126,146,147}, suggesting that Hfq or Hfq-like proteins operate as a hub for post-transcriptional regulation in many diverse bacteria.

Box 2**The enigmatic carboxyl terminus of Hfq**

In many bacterial species, Hfq has carboxy-terminal extensions of >100 amino acids in addition to the conserved core^{148,149}. These diverse C termini are predicted to be unstructured, and most of the available high-resolution structures of Hfq lack this tail (see Supplementary information S1 (table)). The C-terminal tail of *E. coli* Hfq comprises roughly one-third of the protein, and solution X-ray scattering analysis of the full-length protein provides a low-resolution molecular envelope that resembles a six-legged starfish in which the termini occupy the lengthy radial arms in an extended conformation¹⁵⁰. This finding is consistent with the crystal structure of the full-length *E. coli* Hfq, for which the terminus is resolved only as far as residue 74 and is disordered thereafter¹⁵¹.

As the C-terminal tail is positively charged, it could increase protein affinity for RNA, perhaps especially for structured species, for which it could track along exposed grooves in the nucleic acid and form electrostatic interactions with the phosphate backbone. The ensemble of six flexible tails on the hexameric Hfq could also help in finding RNAs¹⁵² by effectively increasing the capture radius of the protein ('fly-casting')¹⁵³. This property could accelerate the on rate and the exchange of sRNAs and mRNAs on Hfq.

As it stands, the importance of the C-terminal tail of Hfq is a matter of controversy: one study has shown tail-less *E. coli* Hfq to be proficient in sRNA binding *in vitro* and its overexpression to have little if any impact on target mRNA regulation *in vivo*¹⁵⁴. By contrast, prior studies had suggested that a lack of the tail reduced bacterial fitness and was associated with defects in sRNA-mediated responses in *E. coli*, probably owing to a severely compromised RNA-binding capacity of the tail-less protein^{152,155}. The controversy notwithstanding, the fact that the Hfq proteins in several bacterial species have maintained extraordinarily lengthy tails in the course of evolution argues that this portion of the protein endows a beneficial function. More studies are clearly needed; for example, RNA sequencing of co-immunoprecipitates¹⁰ could compare the RNAs that are associated with wild-type and tail-less Hfq. Another topic ripe for exploration is whether the C terminus fosters interactions with potential partner proteins of Hfq.

Figure 1. Widely accepted modes of Hfq activity

a | Hfq in association with a small RNA (sRNA) may sequester the ribosome-binding site (RBS) of a target mRNA, thus blocking binding of the 30S and 50S ribosomal subunits and repressing translation. **b** | In some mRNAs, a secondary structure in the 5' untranslated region (UTR) can mask the RBS¹⁵⁶ and inhibit translation. A complex formed by Hfq and a specific sRNA may activate the translation of one of these mRNAs by exposing the translation initiation region for 30S binding^{7,51}. **c** | Hfq may protect some sRNAs from ribonuclease cleavage, which is carried out by ribonuclease E (RNase E) in many cases. **d** |

Hfq may induce the cleavage (often by RNase E^{84,102,103}) of some sRNAs and their target mRNAs. **e** | Hfq may stimulate the polyadenylation of an mRNA by poly(A) polymerase (PAP), which in turn triggers 3'-to-5' degradation by an exoribonuclease (Exo)^{64,157}. In *Escherichia coli*, the exoribonuclease can be polynucleotide phosphorylase, RNase R or RNase II.

Figure 2. The structure of Hfq and its interactions with RNA

a | Secondary-structural elements of the Hfq protomer, highlighting the conserved Sm1 and Sm2 sequence motifs. **b,c** | Each protomer is a compact α - β_{1-5} structural unit (that is, composed of one α -helix and five β -strands) in which the β -strands form a set of antiparallel sheets. One of the strands (β_2) is twisted and curved to such an extent that it contributes to both sheets to form a self-closing, squat barrel (part c). The amino-terminal helix and squat barrel are structural signatures of Hfq-Sm-Sm-like (LSm) proteins and groups them into the wider oligonucleotide-oligosaccharide (OB)-fold structural class, members of which include

the highly conserved single-stranded DNA-binding proteins^{158,159}. The β_4 and β_5 strands on the periphery of each Hfq subunit expose hydrogen-bonding edges that interact with the strands of the neighbouring protomers, so that sheets effectively continue over the entire ring. The organization of secondary-structural elements and protomer–protomer contacts is similar in the hetero-heptameric Sm assembly of the human spliceosome (not shown)²⁹. The inter-strand angles define the spatial relationship of the protomers and, consequently, the number of subunits within the ring. **d** | Two faces for interaction with RNAs (orange) are presented on opposite sides of the Hfq ring. The proximal face (the surface on which the amino-terminal α -helix is exposed) includes residues in the Sm2 sequence motif. Disordered tails are likely to emanate from the equator of the Hfq ring and may form distributive electrostatic interactions with nucleic acids.

Figure 3. A typical Hfq-associated small RNA

a | The domain structure of RybB, a small RNA (sRNA) from *Salmonella enterica*^{46,47}, showing the location of the Hfq-binding region and a potential site of Hfq interaction with the 3' poly(U) tail^{65,66}. The 'seed' region of the sRNA, for mRNA recognition, is shown in an orange box, and the predicted secondary structure of the transcription terminator is the hairpin structure on the right. Hfq also protects regions in the terminator structure from attack by enzymes and chemical probes, suggesting that additional interactions may exist between Hfq and the sRNA. **b** | A comparison of the dimensions of Hfq and RybB sRNA

(according to the *in silico* predicted structure of RybB). This provides an impression of scale as an indication of the potential extent of interaction between Hfq and RNAs.

Figure 4. A three-body problem involving Hfq, small RNA and mRNA

a | Different scenarios envisaged for resolution of the ternary complex formed between Hfq, the small RNA (sRNA) and the target mRNA. An sRNA-mRNA pairing could be the final product (left), the mRNA-Hfq complex could be the most stable product of the encounter (middle), or the ternary complex could bind to other proteins (right; ribonuclease E (RNase E) is shown as a representative effector protein). The schematic is deliberately ambiguous about which face of the Hfq hexamer the RNAs are binding. **b** | A highly speculative energy landscape for the three-body problem. The graph shows the catalytic role of Hfq. Hfq

accelerates the association rate (or on rate), depicted as decreasing the activation barrier for complex formation. It also stabilizes the equilibrium duplex structure of sRNA–mRNA, shown on the right of the reaction coordinate, perhaps by driving metastable structures to a local energy minimum.

Figure 5. A model for RNA exchange on Hfq

The arrangement of multiple RNA-binding sites enables the piecemeal displacement of tightly bound RNA by free RNA. This model accounts for the rapid responses of small RNA (sRNA) function *in vivo*, but it raises the question of how specificity arises in the face of competition from abundant cellular RNAs. Another problem is that sRNAs are not stable in the absence of Hfq, so a large concentration burst of a competing RNA would be required *in vivo* to favour exchange. Figure is modified, with permission, from REF. 38 © (2010) Cold Spring Harbor Laboratory Press.