Critical Reviews in Microbiology, 2012; Early Online: 1–24
© 2012 Informa Healthcare USA, Inc.
ISSN 1040-841X print/ISSN 1549-7828 online
DOI: 10.3109/1040841X.2012.664540

informa
healthcare

REVIEW ARTICLE

The bacterial protein Hfq: much more than a mere RNA-binding factor

Patricio Sobrero and Claudio Valverde

Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña, Bernal, Argentina

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

Abstract
Most of the sequenced bacterial genomes contain a gene encoding a protein known as Hfq that resembles the eukaryotic RNA-binding proteins of the LSm family. It was originally identified in *Escherichia coli* as a host factor required for replication of the Qβ RNA phage. In this review, we present a comprehensive summary of 40 years of investigation to learn that Hfq is an influential, though not essential, global regulator of gene expression in bacteria and that this feature is undoubtedly linked to Hfq's RNA-binding properties. This protein intervenes in different RNA transactions, notably the promotion of antisense interactions between messenger RNAs and small regulatory RNAs. Yet, several aspects of its molecular mechanism remain not understood. In addition, mechanistic studies have been exclusively carried out in enterobacterial models, highlighting the need to expand the research on Hfq function to other taxons. Upon reviewing the genetic, structural, biochemical, and biological aspects of this extraordinary protein, we discuss recent findings on interactions with macromolecules other than RNA suggesting a broader participation of Hfq in major steps in the flow of genetic information. We show that, although significant progress has been achieved to elucidate Hfq role at the molecular level, many open questions remain.

Keywords: Hfq, Sm-protein, nucleic acid binding protein, sRNA–mRNA interactions, global regulator

Copyright © 2012 Informa Healthcare USA, Inc. Not for Sale or Commercial Distribution. Unauthorised use prohibited. Authorised users can download, display, view and print a single copy for personal use

Riboregulation and small regulatory RNAs

The regulation of gene expression is not a simple process. Regardless of the greater simplicity of a unicellular organism, bacteria fall within this generalization. Even though the principal mechanism for the regulation of gene expression in bacteria generally occurs at the transcriptional level by means of regulatory polypeptides, bacteria have evolved complex networks of regulation at different levels in the processing of genetic information. This approach allows bacterial cells to generate precise individual responses in order to improve their fitness to the environment and especially their economy of the resources. During the past two decades, posttranscriptional regulatory mechanisms have been recognized as being fairly widespread in bacteria, and as such contributing in varying degrees to the fine tuning of gene expression. In particular, ribonucleic acids have emerged as the central key players in posttranscriptional networks, particularly with respect to a fast and concerted regulatory response under stressful conditions.

The term riboregulation stands for regulatory mechanisms of gene expression that are based on the functioning of RNA molecules (Papenfort and Vogel, 2010). This role includes *cis*-encoded regulatory-RNA elements such as riboswitches, thermosensors, and attenuators as well as independently encoded transcripts that interact with other transcripts (usually messenger RNAs [mRNAs]). At all events, a given form of regulation may result in changes in the rate of mRNA-transcript elongation, the efficiency of mRNA translation, and/or the stability of mRNA. In addition, a given mechanism of regulation may depend solely on the regulatory RNA element involved or may also require protein factors, such as Hfq or members of the CsrA/RsmA translational regulators (Babitzke et al., 2009). The recent development and application of high-throughput RNA sequencing has revealed that a significant proportion of bacterial transcriptional activity consists of untranslated RNA molecules (Schluter et al., 2010; Sharma et al., 2010; Mitschke et al., 2011). Two major classes of

Address for Correspondence: Claudio Valverde, Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Roque Saenz Peña 352, Bernal B1876BXD, Argentina. Tel: +54-11-4365-7100 ext 4313. Fax: +54-11-4365-7132. E-mail: cvalver@unq.edu.ar

(Received 08 November 2011; revised 27 January 2012; accepted 03 February 2012)

RIGHTSLINK

noncoding-RNA transcripts are the *cis*-antisense RNAs (asRNAs) and the trans-encoded–small regulatory RNAs (sRNAs). Although mRNA–asRNA pairing may also be assisted by Hfq (Lorenz et al., 2010; Berghoff et al., 2011), this review will focus on the role of Hfq solely in sRNA-mediated regulation (Figure 1). For a comprehensive review about the properties of asRNAs, we refer the reader to the study by Georg and Hess (2011).

sRNA molecules are single transcripts of about 50–300 nt encoded in intergenic regions. Till date, nearly 150 sRNAs have been detected in *Escherichia coli*. This represents more than 3% of the sequences of all the protein-encoding genes (Huang et al., 2009; Raghavan et al., 2011). Systematic computational and transcriptomic searches in other bacterial models whose genomes had already been sequenced revealed a comparable figure (Livny et al., 2006; Coenye et al., 2007; Valverde et al., 2008; Liu et al., 2009; DiChiara et al., 2010; Postic et al., 2010; Schluter et al., 2010; Raghavan et al., 2011). Bacteria would thus appear to count on a large number of sRNAs to modulate gene expression. sRNA expression is itself regulated and can be triggered by different kinds of signals: for example, *ryhB* is induced under iron limitation (Masse et al., 2003) and *sgrS* upon phosphosugar stress (Vanderpool and Gottesman, 2004), whereas *micA* and *rybB* respond to membrane stress (Papenfort et al., 2006; Johansen et al., 2008). Theoretical models support the notion that the utilization of sRNAs enables a prompt regulatory response compared with protein-based control, as the synthesis of an sRNA is faster and more direct than that of a protein (Shimoni et al., 2007; Mehta et al., 2008). Furthermore, sRNA levels often decay rapidly once the signal is absent because of their high turnover rate (Masse et al., 2003; Viegas and Arraiano, 2008) or due to codegradation of mRNA–sRNA duplexes (Aiba, 2007). This allows an appropriately transitory response in a changing environment.

In terms of mechanism, most of the sRNAs studied thus far base pair with their target mRNAs to control the translation and/or the stability of the mRNA (Beisel and Storz, 2010). This union usually imposes a negative regulation on mRNA translation since base pairing takes place in the vicinity of the ribosome-binding site (Maki et al., 2010) or even downstream within the coding sequence (Pfeiffer et al., 2009). In some cases, sRNA–mRNA duplex formation and translational blockage results in accelerated degradation of the target mRNA or both RNA molecules, upon Hfq-dependent recruitment of RNAse E (Aiba, 2007, Caron et al., 2010). In this way, mRNA silencing becomes irreversible. Nevertheless, several examples of direct positive control by sRNAs have also been reported; this mechanism involves the pairing of the activator sRNA to its target mRNA (Frohlich and Vogel, 2009). In most of the reported cases, the sRNA–mRNA interaction precludes the formation of a secondary structure that inhibits translation and thereby prevents mRNA repression (Prevost et al., 2007; Soper et al., 2010). Increased translation may also occur through mRNA stabilization

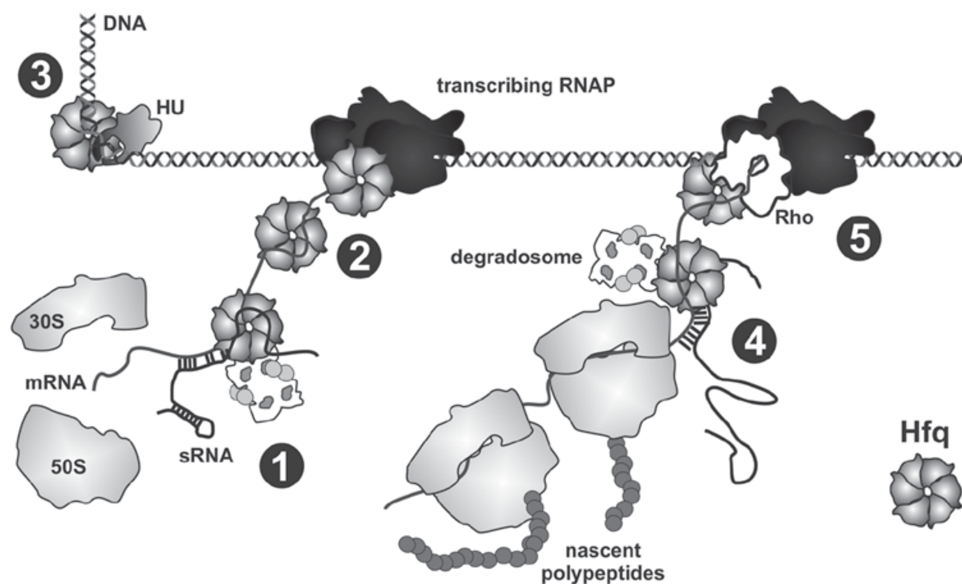


Figure 1. Involvement of the global regulatory protein Hfq in the control of gene expression by different mechanisms at different levels of the genetic information flow. (1) Hfq promotes antisense pairing between a small regulatory RNA (sRNA) and a target messenger RNA (mRNA), usually near or at the ribosome-binding site that impairs ribosomal access. Hfq recruits the degradosome, facilitating coupled mRNA and sRNA degradation and thus silencing mRNA expression. (2) As an RNA chaperone, Hfq may locally affect mRNA folding and attenuate transcriptional pausing. RNA-substrate recognition by Hfq may occur directly at the site of transcription since the RNA–polymerase holoenzyme copurifies with Hfq. (3) Hfq is able to bind DNA and also copurify the histone-like proteins HU. Thus, a role in DNA bending and transcriptional control could be possible. (4) Hfq- and sRNA-mediated translational control within the coding region of an mRNA may uncouple translation from transcription and facilitate Rho-dependent termination. (5) In the absence of translational control, Hfq may control antitermination through its interaction with Rho. See text for further details and references.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

at the 3′-end upon sRNA binding to the 3′-UTR (Opdyke et al., 2004).

A single sRNA may target several mRNAs, as is the case of the well-characterized global regulatory sRNAs GcvB (Sharma et al., 2007), OmrA/B (Guillier and Gottesman, 2008), RsaE (Geissmann et al., 2009), and RybB (Papenfort et al., 2010). The multitarget action is a key feature of the sRNA-based regulation: the expression of several genes and operons can be tuned and synchronized through a sole sRNA pathway. Antisense sRNA–mRNA interactions involve short and discontinuous regions of complementarity, starting with a seed stretch of as few as 7 bp (Balbontin et al., 2010). In order for this interaction to take place in the highly chaotic milieu of a living cell, additional factors are required to ensure the specificity and magnitude of the response over an appropriate time scale. Thus far, the only factor described to have such a functional role in facilitating the interaction between sRNAs and its mRNA target is the protein Hfq (Figure 1).

Hfq: from a host factor for coliphage replication to a global regulator of RNA interactions

The history of Hfq began in 1968, when Franze de Fernandez and collaborators discovered the requirement of a couple of *E. coli* cellular factors for *in-vitro* replication of the RNA phage Qβ (Franze de Fernandez et al., 1968). One of these agents, HF_I, was purified and partially characterized as a hexameric protein able to bind different single-stranded RNAs, but not double-stranded RNA or DNA (Franze de Fernandez et al., 1972). The subsequent studies focused primarily on the protein's binding properties (Senear and Steitz, 1976; de Haseth and Uhlenbeck, 1980b; de Haseth and Uhlenbeck, 1980a). Only by the early 1990s was the *E. coli* gene encoding HF_I identified and designated *as hfq* (Kajitani and Ishihama, 1991). Since then, the attention has shifted to the role that Hfq has in the physiology and control of gene expression within the bacterial cell.

Hfq is a phylogenetically widespread protein, although not ubiquitous (Figure 2; Supp. Table 1). The rapidly

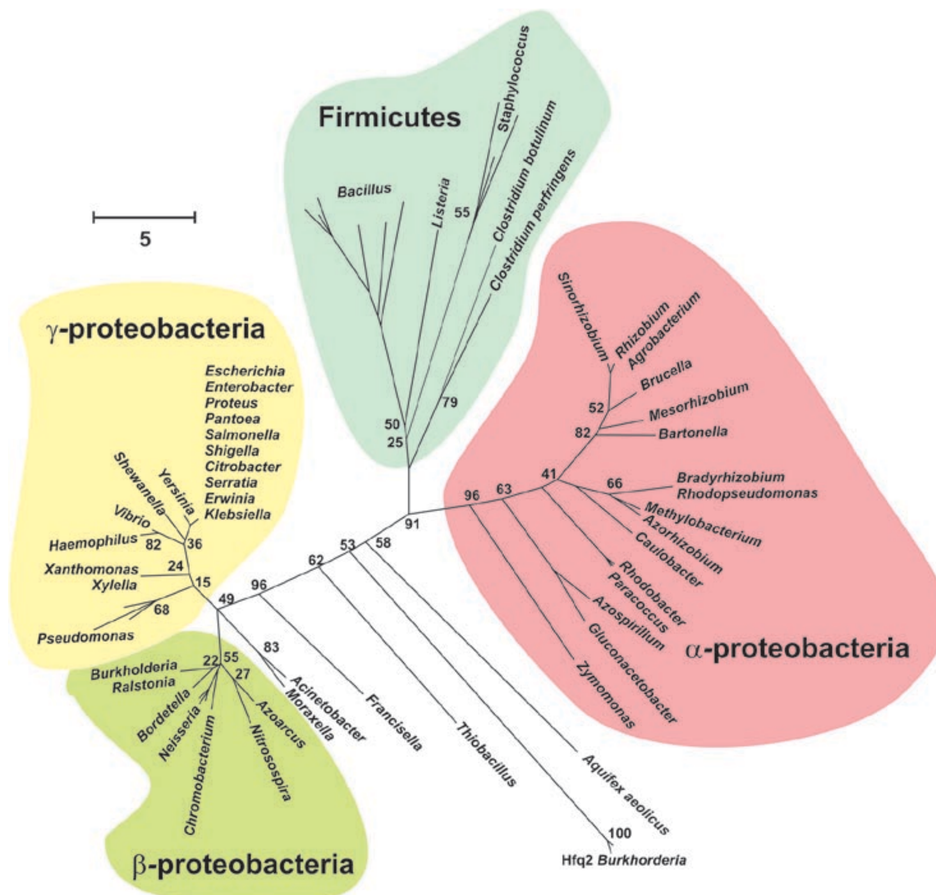


Figure 2. Phylogenetic relationships among bacterial Hfq proteins. The evolutionary history depicted was inferred through the neighbor-joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 271 665 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is presented next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Those evolutionary distances were computed by means of the number-of-differences method (Nei and Kumar, 2000) and are in the units of number of amino acid differences per sequence. The analysis involved 100 amino acid sequences (the multiple alignment for these sequences is shown in the Supp. Fig. 1). All positions containing gaps and missing data were eliminated. A total of 63 positions were present in the final data set. The evolutionary analyses were conducted in the MEGA5 software (Tamura et al., 2011). (See colour version of this figure online at www.informahealthcare.com/mby)

© 2012 Informa Healthcare USA, Inc.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

Table 1. Phenotypes of bacterial *hfq* mutants.

Microorganism	Taxon	Ecological niche	Type of mutant	Phenotypes	Phenotype complemented by *E. coli hfq*	References
Azorhizobium caulinodans[a]	α-proteobacteria	Legume (Sesbania) stem nodules: Nitrogen-fixing symbiont	Insertional (hfq::ΩKm^R)*	Cannot grow under nitrogen-fixing conditions; reduced nifA expression	Yes (partially)	Kaminski et al., 1994; Kaminski and Elmerich, 1998
Brucella abortus[b]	α-proteobacteria	Facultative intracellular pathogen: Causes brucellosis	Insertional (hfq:: ΩKm^R)*	Reduced survival in stationary phase and within macrophages, higher sensitivity to H_2O_2 and acid stress in stationary phase; reduced virulence in mice	n.d.	Robertson and Roop 1999
Rhizobium leguminosarum biovar *viciae*[a]	α-proteobacteria	Legume (pea) root nodules: Nitrogen-fixing symbiont	Spontaneous; single-base replacement	Suppression of impaired amino acid transport associated with glutamate synthase (gltB) mutation	n.d.	Mulley et al., 2011
Rhodobacter capsulatus[a]	α-proteobacteria	Purple nonsulfur photosynthetic and nitrogen-fixing bacterium	Insertional (hfq:: ΩSp^R)*	Reduced growth under nitrogen-fixing conditions, reduced nitrogenase activity and *nif* gene expression	n.d.	Drepper et al., 2002
Rhodobacter sphaeroides[a]	α-proteobacteria	Facultative phototrophic and nitrogen-fixing bacterium	Insertional (hfq:: ΩSp^R)*	Asymmetrical cell division; reduced bacteriochlorophyll content; reduced tolerance to oxidative stress	n.d.	Berghoff et al., 2011
Sinorhizobium meliloti[a]	α-proteobacteria	Legume (alfalfa) root nodules: Nitrogen-fixing symbiont	Insertional deletion (Δhfq::lacZ)*;In frame deletion (Δhfq)	Reduced symbiotic efficiency; reduced growth at neutral and alkaline pH; reduced motility; enhanced exopolysaccharide production	Yes (partially)	Barra-Bily et al., 2010a; Barra-Bily et al., 2010b; Torres-Quesada et al., 2010; Sobrero and Valverde, 2011
Burkholderia cenocepacia[b]	β-proteobacteria	Opportunistic human pathogen: Causes pneumonia and is associated with cystic fibrosis (lungs)	In frame deletion (Δhfq2) and silenced hfq	Reduced virulence on *Caenorhabditis elegans*; reduced tolerance to acid, heat, oxidative and saline stress	n.d.	Ramos et al., 2011
Burkholderia cepacia[b]	β-proteobacteria	Opportunistic human pathogen: Causes pneumonia and is associated with cystic fibrosis (lungs)	Insertional (hfq:: ΩTp^R)*	Reduced colonization and virulence on *Caenorhabditis elegans*	Yes	Sousa et al., 2010
Neisseria gonorrhoeae[b]	β-proteobacteria	Human pathogen: Causes the sexually transmitted disease—gonorrhea	Insertional (hfq:: ΩEr^R)	Reduced growth rate, reduced adherence to epithelial cells. Slight changes in virulence and inflammatory response of epithelial cells	n.d.	Dietrich et al., 2009

(*Continued*).

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.
RIGHTSLINK

Table 1. (Continued).

Microorganism	Taxon	Ecological niche	Type of mutant	Phenotypes	Phenotype complemented by *E. coli hfq*	References
Neisseria meningitidis[b]	β-proteobacteria	Human pathogen: Causes meningitis	Insertional deletion (Δhfq:: ΩCm^R); Insertional deletion (Δhfq:: ΩKm^R)	Reduced growth and plating efficiency; reduced tolerance to membrane, oxidative, or saline stress, and to polymyxin B; reduced survival in human blood and increased serum sensitivity; reduced colonization of mice after intraperitoneal inoculation	n.d.	(Fantappie et al., 2009; Mellin et al., 2010)
Acinetobacter baylyi[a]	γ-proteobacteria	Nonpathogenic soil bacterium	Insertional (hfq:: ΩSp^R)	Reduced growth rate and yield, loss of cell chain formation	n.d.	Schilling and Gerischer, 2009
Escherichia coli K12[a]	γ-proteobacteria	Laboratory model strain	Insertional (hfq:: ΩKm^R)*	Reduced plating efficiency of coliphage Qβ; reduced growth rate and yield, increased cell size, reduced tolerance to osmotic stress, UV radiation and increased oxidation of various C sources	n.d.	Franze de Fernandez et al., 1972; Tsui et al., 1994)
Escherichia coli O157:H7[b]	γ-proteobacteria	Enterohemorragic human pathogen	In frame deletion (Δhfq)	Reduced tolerance to acid stress; reduced swimming motility; premature virulence factor expression and lesions in HeLa cells in exponential phase	Yes	Hansen and Kaper, 2009
Escherichia coli UTI89[b]	γ-proteobacteria	Uropathogenic human pathogen	Insertional (hfq:: ΩCm^R)*	Reduced colonization of the mice urinary tract; reduced swimming motility, tolerance to acid stress, polymyxin B, and nitrogen- and oxygen-reactive species; reduced biofilm formation	Yes	Kulesus et al., 2008
Francisella novicida[b]	γ-proteobacteria	Mouse pathogen: Causes tularemia in mice	In frame deletion (Δhfq)	Reduced growth; reduced tolerance to saline, acid and oxidative stress; increased biofilm formation	n.d.	Chambers and Bender, 2011
Francisella tularensis[b]	γ-proteobacteria	Human pathogen: Causes tularemia	In frame deletion (Δhfq)	Reduced growth; reduced tolerance to saline and membrane stress; attenuated virulence in mice	n.d.	Kadzhaev et al., 2009; Meibom et al., 2009

(*Continued*).

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

Table 1. (Continued).

Microorganism	Taxon	Ecological niche	Type of mutant	Phenotypes	Phenotype complemented by *E. coli hfq*	References
Klebsiella pneumoniae[b]	γ-proteobacteria	Human pathogen: Causes a range of diseases, from urinary tract infections to life-threatening systemic infections	Insertional deletion (Δhfq::ΩCm^R)*	Reduced dissemination into extra-intestinal organs and attenuated induction of a systemic infection in a mouse model. Reduced tolerance to oxidative, thermal, and UV stress.	n.d.	Chiang et al., 2011
Legionella pneumophila[b]	γ-proteobacteria	Human intracellular pathogen: Causes legionnaires' disease	Deletion (?)*	Reduced growth at 30°C and 37°C; slight defects in virulence in both amoeba and macrophage infection models	n.d.	McNealy et al., 2005
Moraxella catarrhalis[b]	γ-proteobacteria	Opportunistic human pathogen: Causes otitis media and infectious exacerbations of chronic obstructive pulmonary disease	In frame deletion (Δhfq)	Reduced growth; reduced tolerance to saline and oxidative stress	n.d.	Attia et al., 2008
Pseudomonas aeruginosa[b]	γ-proteobacteria	Opportunistic human pathogen: Associated with cystic fibrosis (lungs) and skin burns.	Insertional (hfq:: ΩSp/Sm^R)*	Attenuated virulence in mice (intraperitoneal) and *Galleria mellonella* larvae; reduced growth rate, reduced elastase, catalase, and pyocyanin production; impaired type IV pili-dependent twitching and swarming motility	n.d.	Sonnleitner et al., 2003
Pseudomonas fluorescens 2P24[a]	γ-proteobacteria	Plant probiotic bacteria: Antagonizes pathogenic fungi.	Insertional (hfq::miniTn5) *	Reduced colonization of wheat root; reduced biofilm formation, antibiotic synthesis, and quorum-sensing signal production	n.d.	Wu et al., 2010
Salmonella enterica serovar Typhimurium[b]	γ-proteobacteria	Human pathogen: Causes salmonellosis and enteric fever	Insertional deletion (Δhfq::ΩCm^R)	Reduced virulence in mice (upon oral or intraperitoneal infection), reduced replication in macrophages, reduced host cell adhesion and invasion (in vitro), defective secretion of effector proteins, chronic envelope stress, reduced motility	n.d.	Sittka et al., 2007

(*Continued*).

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

Table 1. (Continued).

Microorganism	Taxon	Ecological niche	Type of mutant	Phenotypes	Phenotype complemented by *E. coli hfq*	References
Shigella sonnei	γ-proteobacteria	Human pathogen: Causes shigellosis	In frame deletion (Δhfq)	Reduced survival in stationary phase; attenuated virulence in guinea pig keratoconjunctivitis model; increased TTSS-dependent invasion of HeLa cells	n.d.	Mitobe et al., 2009
Vibrio alginolyticus[b]	γ-proteobacteria	Fish pathogen: Causes vibriosis	In frame deletion (Δhfq)	Attenuated virulence in zebra fish; reduced tolerance to osmotic stress, ethanol, temperature shift, and iron starvation; reduced motility and biofilm formation	n.d.	Liu et al., 2011
Vibrio cholerae[b]	γ-proteobacteria	Human enteropathogen: Causes cholera	In frame deletion (Δhfq)	Reduced mice intestinal colonization; slight reduction in growth rate and yield.	n.d.	Ding et al., 2004
Vibrio parahaemolyticus[b]	γ-proteobacteria	Human enteropathogen: Causes gastroenteritis	In frame deletion (Δhfq)	Reduced growth; increased hemolytic activity	n.d.	Nakano et al., 2008
Yersinia pestis biovar *Microtus*[b]	γ-proteobacteria	Facultative intracellular mice pathogen: Avirulent in humans	Insertional deletion (Δhfq::ΩKm^R) *	Attenuated virulence in mice after subcutaneous or intravenous infection; reduced growth in minimal medium, reduced tolerance to H_2O_2, heat and polymyxin B; reduced resistance to phagocytosis and survival within macrophages.	n.d.	Geng et al., 2009
Yersinia pestis KIM10[b]	γ-proteobacteria	Human pathogen: Causative agent of plague	Insertional deletion (Δhfq::ΩCm^R)*	Reduced growth at 37 °C; elongated cells.	n.d.	Bai et al., 2010
Yersinia pseudotuberculosis[b]	γ-proteobacteria	Mild human enteropathogens: Causes gastroenteritis	In frame deletion (Δhfq)	Attenuated virulence in mice intragastric infection model and upon intraperitoneal infection; reduced intracellular survival in macrophages; increased motility and production of a biosurfactant-like substance	n.d.	Schiano et al., 2010

(*Continued*).

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

Table 1. (Continued).

Microorganism	Taxon	Ecological niche	Type of mutant	Phenotypes	Phenotype complemented by *E. coli hfq*	References
Borrelia burgdorferi	Spirochaetales	Human pathogen: Causes Lyme disease	Insertional deletion (Δhfq:: ΩCm[R])*	Reduced growth rate, increased cell length, loss of virulence in mice upon needle inoculation	Yes	Lybecker et al., 2010
Synechocystis sp. PCC 6803[a]	Cyanobacteria	Free-living autotrophic and nitrogen-fixing bacteria	Insertional (hfq:: ΩCm[R])	Loss of phototactic response, type IV pili, and natural transformability	n.d.	Dienst et al., 2008
Listeria monocytogenes[b]	Firmicutes	Human food-borne pathogen: Causes listerioris	In frame deletion (Δhfq)	Reduced tolerance to salt and ethanol stress, reduced viability under amino acid starvation, and reduced intraperitoneal survival in mice	Yes	Christiansen et al., 2004, Nielsen et al., 2010
Staphylococcus aureus[b]	Firmicutes	Human opportunistic pathogen	Insertional (hfq:: ΩCm[R])	No evident phenotypes in stress-resistance tests and virulence gene expression	n.d.	Bohn et al., 2007
Staphylococcus aureus[b]	Firmicutes	Human opportunistic pathogen	Insertional deletion (Δhfq:: ΩKm[R])	Increased carotenoid pigment production, associated with increased tolerance to oxidative stress; attenuated toxicity during infection of MDBK cell and a mouse model of peritonitis	n.d.	Liu et al., 2010

Abbreviations: n.d., not determined; (?), not specified. TTSS, type three secretion system.
[a]Corresponds to free-living saprophytes or probiotic bacteria for plants or animals.
[b]Corresponds to pathogenic bacteria.
*The mutation, considering the genetic context and the construction strategy, may have polar effects on downstream co-transcribed genes.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

growing database indicates that *hfq* sequences are present in many bacterial taxons (Figure 2; Supp. Table 1) and in a few archaeal species (Nielsen et al., 2007). Thus, we can assume an ancient origin for Hfq. This protein seems, however, to be absent in those microorganisms that have undergone genome reduction upon shifting to an intracellular way of life (e.g., *Rickettsia*, *Chlamydia*, or *Buchnera*) or that have lost the *hfq* gene through other unknown evolutionary constraints (Supp. Table 1). Till date, all sequenced ε-proteobacteria, like *Helicobacter pylori* and *Campylobacter jejuni,* lack *hfq* homologs. The same statement applies to actinomicetales, like *Frankia* and *Streptomyces* (Supp. Table 1). As sRNAs have been identified in some of these microorganisms lacking *hfq* (Swiercz et al., 2008; Arnvig and Young, 2009; Sharma et al., 2010; Tsui et al., 2010), one possible explanation would be that, as in cyanobacteria, the Hfq structure has been more conserved than the polypeptide sequence (Boggild et al., 2009). This could explain why *in-silico* similarity searches have failed to detect the corresponding homolog. Alternatively, Hfq's function may be dispensable (Bohn et al., 2007) or else dependent on other unidentified host factors (Pandey et al., 2011).

The role of Hfq in the physiology of bacteria has been explored in several model organisms, from human pathogens to plant symbionts. In the majority of the strains studied, *hfq* has been knocked out (Table 1), whereas in a few others, the gene has been identified as part of a search for candidate genes regulating a phenotype of interest (Wu et al., 2010; Mulley et al., 2011). In most of the examples, the absence of Hfq results in pleiotropic phenotypic alterations that compromise the fitness of the bacteria and the responses against stressful environmental conditions (summarized in Table 1). Notably, the virulence of pathogenic bacteria is attenuated if *hfq* has been deleted or interrupted (Table 1). Because of the

RIGHTSLINK

nature of some of the mutations, however, the possibility that certain phenotypes could result from polar effects on downstream genes cannot be discarded (Table 1). Nevertheless, the pleiotropic changes of *hfq*-deletion mutants argue for a global role of Hfq in the physiology of the bacterial cell. This conclusion is independently supported by molecular studies showing the stringent and specific association of sRNAs with the Hfq protein (Zhang et al., 2003; Christiansen et al., 2006; Sittka et al., 2009; Berghoff et al., 2011; Olejniczak, 2011). We would thus expect that a cell devoid of Hfq would have serious problems executing sRNA-based regulatory mechanisms in response to different environmental stimuli (Table 1).

Regulation of hfq expression

The gene product Hfq is involved in the global regulation of gene expression, but the question here is whether or not *hfq* expression itself is regulated. In *E. coli*, Hfq is an abundant protein whose level seems to respond moderately to the cellular growth rate (Kajitani et al., 1994; Vytvytska et al., 1998) and to the growth phase (Kajitani et al., 1994). Kajitani and colleagues published an estimate of 5000–10 000 Hfq oligomers per cell for log phase *E. coli* growing in M9-glucose (Kajitani et al., 1994). Even though most of Hfq was recovered from the cytoplasmic fraction, 10–20% was found in association with the nucleoid fraction, suggesting a direct interaction of Hfq with chromosomal DNA (Kajitani et al., 1994). A pool of Hfq oligomers associated with the membrane might, however, exist as well (Diestra et al., 2009). The estimation of Hfq intracellular concentration by quantitative western blots showed a growth phase-dependent variation in Hfq concentration, from 10 000 hexamers per cell in the log phase to 5000 hexamers per cell in the stationary phase in LB medium (Ali Azam et al., 1999). This decrease in Hfq concentration, which resembles the regulatory pattern of growth-related genes such as those encoding the RNA polymerase (RNAP), was also noted previously by Kajitani and collaborators (Kajitani et al., 1994). The Hfq rate synthesis is under the control of cell growth rate, as has been found for the protein components of the transcription and translation apparatus (Ishihama 1976; Liveris et al., 1991), suggests that Hfq is one of the essential components for cell growth. In fact, a shared phenotype of most strains with disrupted *hfq* is a reduction in their growth rate (Table 1). Recent large-scale studies of the *E. coli* cytosolic proteome at exponential growth phase validated the cellular abundance of Hfq with an estimate of 5800 molecules per cell, thus positioning Hfq within the group of 179 highly abundant proteins at more than 2000 copies per cell—with ribosomal proteins S8, S9, and L11; elongation factor P; and the Rho-termination factor having comparable abundances (Ishihama et al., 2008). The quantitation by Ishihama and coworkers may underestimate the true size of the Hfq pool because only the cytoplasmic fraction has been analyzed, possibly losing the protein fraction that could be associated with the nucleoid and/or the membrane. Consequently, a more accurate estimation of Hfq intracellular concentration and of its functional distribution is required in order to refine our understanding of the role of Hfq in the regulation of gene expression.

The gene *hfq* is part of the *E. coli* superoperon *amiB–-mutL–miaA–hfq–hflX–hflK–hflC*, which genetic sequence has a complex transcriptional organization; containing three σ32-dependent heat-shock promoters in addition to four σ70-dependent promoters (Tsui et al., 1996). In addition, RNAse E is involved in the control of *hfq*-transcript levels, as that species accumulates 3-fold in an *rne* mutant over the levels in the wild type (Tsui and Winkler, 1994). An inspection of the *hfq* genetic context within bacterial genomes reveals an interesting concordance. The *amiB–mutL–miaA–hfq–hflX–hflK–hflC* superoperon is well conserved only in most γ-proteobacteria. In the α- and β-proteobacteria, as well as in some bacillales, the synteny is restricted to the *hfq*-*hflX* tandem (Supp. Table 2). In these bacteria, the *hflX* ORF is always located a few nucleotides downstream from *hfq*, suggesting that they are cotranscribed. The cotranscription, however, has been shown in only *E. coli* (Tsui and Winkler, 1994), *Francisella tularensis* (Meibom et al., 2009), and *Sinorhizobium meliloti* (Sobrero and Valverde, 2011). As the function of HflX still remains poorly characterized, we continue to ignore whether or not the transcriptional linkage between *hfq* and *hflX* that is reflected as synteny (Supp. Table 2) corresponds to a participation of those genes in biological processes that are functionally related. The *hflX* gene encodes a GTPase, an enzyme that can associate with the ribosomal 50S subunit, but not with the 30S one; and this interaction stimulates the enzyme's activity (Shields et al., 2009). The relatively low number of HflX molecules per *E. coli* cell (<200; Ishihama et al., 2008), however, indicates that this interaction is not stoichiometric and also may be temporary. HflX also has ATPase activity, but in contrast to HflK and HflC, this protein is not involved in the λ-phage replication cycle, or in other transposition processes (Dutta et al., 2009). HflX may somehow be involved in the RNA metabolism of the bacterial cell since the protein interacts with the translation machinery. One possibility is that HflX plays a role as a translation-factor–related GTPase (Dutta et al., 2009).

The expression of *hfq* has also been studied in *Staphylococcus aureus*. This protein has been immunodetected in some strains but not in others is noteworthy, although the *hfq* mRNA was present in all the *S. aureus* strains studied (Liu et al., 2010). Thus, for certain strains, *hfq* expression would seem to fail at the translational level, which deficiency explained the lack of detectable phenotypes associated with *hfq* deletions in those strains (Bohn et al., 2007).

In addition to the *hfq* transcriptional features described above, strong evidence exists for translational regulation. For *E. coli* and *S. meliloti*, two bacterial species that diverged long ago in the tree of life (Figure 2), Hfq has been demonstrated to be able to control its own

© 2012 Informa Healthcare USA, Inc.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

translation (Vecerek et al., 2005; Sobrero and Valverde, 2011). In the γ-proteobacterium *E. coli*, Hfq binds to its own mRNA leader at two different regions. Such binding of Hfq represses *hfq* translation since Hfq competes with the translation machinery for the ribosome-binding site (Vecerek et al., 2005). In the γ-proteobacterium *S. meliloti*, Hfq controls the expression of an *hfq´–´lacZ* translational fusion, and this process requires only the presence of the Hfq protein (Sobrero and Valverde, 2011). Whether sRNAs targeting the *hfq* mRNA leader contribute *in vivo* to the translational regulation of *hfq* observed in *E. coli* and *S. meliloti* is not clear. Furthermore, in the photosynthetic γ-proteobacterium *Rhodobacter sphaeroides*, the *hfq* mRNA was identified within the pool of RNA that coimmunoprecipitated with Hfq, which indirectly suggests an autoregulatory process (Berghoff et al., 2011). At all events, the evidence for the translational autocontrol of Hfq levels highlights two significant features. The first is the evolutionary conservation of this process, which would be expected eventually to be observed in other bacteria. The second is the delicate control of *hfq* expression to ensure that the Hfq protein concentration is kept within a limited range. This issue is not minor; if Hfq levels remain constant, the changes in sRNA concentration will come to the forefront in the control of riboregulatory processes (Adamson and Lim, 2011; Moon and Gottes Man, 2011). A reinforcement of the data on Hfq concentration inside the cell would thus be essential in order to obtain a more detailed understanding of the biochemistry and biophysics of the RNA transactions that are assisted by this molecular chaperone.

Structural features of Hfq

In order to survey the role of Hfq in the physiology of bacteria, we have to understand how this protein functions; and this understanding involves a familiarization with the Hfq structure. The Hfq monomer in bacteria is a rather small polypeptide ranging from 8 to 11 Kda, with this molecular weight variation involving the C-terminal region (Figure 3; Supp. Fig. 1). Despite this small size, the core sequence is highly conserved (Figure 3; Supp. Fig. 1), and this strong primary sequence conservation extends to the elements of this protein's secondary structure as well as to the three-dimensional structure (Figure 4).

Fundamental hints on the three-dimensional structure of Hfq were first provided almost 10 years ago by a series of parallel works involving *in-silico* homology-based modeling (Arluison et al., 2002; Sun et al., 2002), electron microscopy (Zhang et al., 2002), and crystallography (Schumacher et al., 2002). At present, 19 structures of hexameric Hfq proteins are deposited in the PDB protein data bank: five structures from *E. coli* (Sauter et al., 2003; Link et al., 2009; Beich-Frandsen et al., 2011b; Wang et al., 2011), two from *S. aureus* (Schumacher et al., 2002), two from *Salmonella typhimurium* (Sauer and Weichenrieder, 2011), four from *Pseudomonas aeruginosa* (Nikulin et al., 2005; Moskaleva et al., 2010), two from the cyanobacteria *Anabaena* and *Synechocystis* (Boggild et al., 2009), two from *Bacillus subtilis* (Baba et al., 2010), and the last ones from the γ-proteobacterium *Herbaspirillum seropedicae* (Kadowaki et al., 2012) and the archaeon *Methanococcus jannaschii* (Nielsen et al., 2007). All these structures share the following features, which have been recently reviewed (Brennan and Link, 2007; Vogel and Luisi, 2011): (1) Hfq proteins are circular hexamers that delimit a central pore (Figure 4); (2) every monomer has an LSm-like fold with a β5-α1-β-β2-β3-β4 topology (Figures 3 and 4); (3) the hexamer presents two asymmetric faces, usually with a net positive electrostatic potential (Figure 4), as expected for a nucleic acid-binding protein; and (4) the N-terminal α-helix is exposed in the so-called proximal face (Figure 4). Finally, it has been recently revealed that the C-terminal region lying beyond the monomer core extends laterally away from the oligomer distal face (Beich-Frandsen et al., 2011a; Beich-Frandsen et al., 2011b).

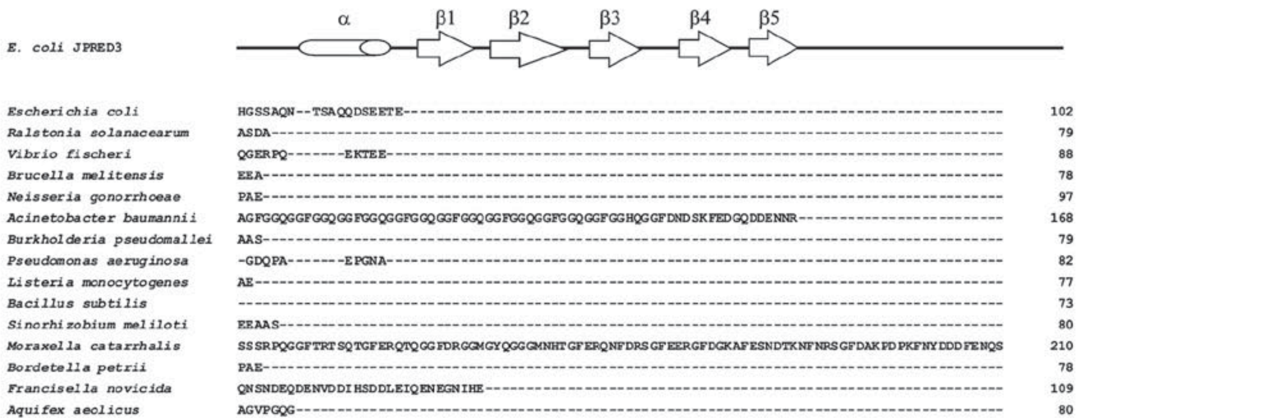
Sequence-homology searches and structural studies positioned Hfq as an LSm-like protein (Moller et al., 2002; Sun et al., 2002; Zhang et al., 2002) (Figures 3 and 4). LSm proteins are eukaryotic agents that participate in ribonucleoprotein complexes such as the spliceosome (Verdone et al., 2004). These proteins tend to form heptameric toroid (i.e., doughnut-shaped) oligomers that expose a highly positively charged surface (Naidoo et al., 2008). The Hfq Sm-1 motif is more similar to that of eukaryotic LSm-proteins than is the Sm-2 motif (Figure 3). The former (Figure 3), for its part, contains several highly phylogenetically conserved residues consisting in hydrophobic amino acids, an acidic aspartate, and a glycine; all involved in the maintenance of the Sm fold. The Sm-2 motif is furthermore unusually conserved among the bacterial Hfq sequences (Figure 3). This motif is composed of several residues that are crucial for the stabilization of the protein structure, such as the histidine located between helices β4 and β5 (Moskaleva et al., 2010). Even the atypical Hfq2 from the *Burkholderia* species—which molecule is approximately 100 amino acids longer than the classical Hfq proteins—possesses the two characteristic Sm motifs (Ramos et al., 2011).

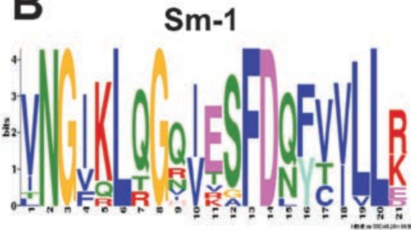
Since Hfq works as an RNA-binding protein, surface properties have to drive the interaction of Hfq with its different targets and, as a consequence, the biophysical mechanisms that determine sRNA–mRNA pairing. These surface properties are facilitated by the net positive electrostatic potential of the two Hfq faces, a feature that seems to be conserved in Hfq homologs (Figure 4). Small differences in the electrostatic distribution over the Hfq surface can lead to an alteration of the RNA-binding capacity. Such a consideration could also explain the different affinities of *Anabaena* and *Synechocystis* Hfq for the Spot42 sRNA (Boggild et al., 2009). The ring-shaped Hfq oligomer possesses two different surfaces (Figure 4), both of which constitute binding sites that can discriminate between RNA molecules (Vogel and Luisi, 2011). The proximal face of *S. aureus* and *E. coli*' Hfq has

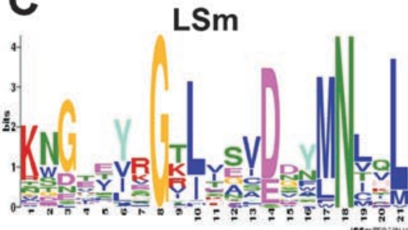
Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.







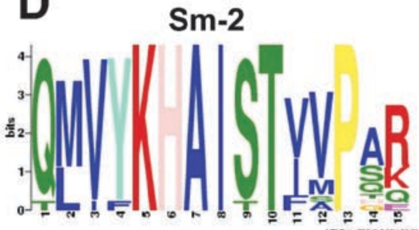


Figure 3. Hfq is an Sm-protein with a conserved sequence core and conserved secondary structure elements. (A) Sequence alignment of Hfq proteins from representative species of different phylogenetic groups. The corresponding accession numbers are as follows: *Escherichia coli* K12 (ACE63256), *Ralstonia solanacearum* (NP_519341), *Vibrio fischeri* (YP_002157119), *Brucella melitensis* (YP_002732845), *Neisseria gonorrhoeae* (ZP_06132667), *Acinetobacter baumannii* (YP_001713357.1), *Burkholderia pseudomallei* (YP_108138.1), *Pseudomonas aeruginosa* (AAG08329.1), *Listeria monocytogenes* (YP_002350237), *Bacillus subtilis* (YP_004207790), *Sinorhizobium meliloti* (NP_385570), *Moraxella catarrhalis* (YP_003627066), *Bordetella petrii* (YP_001630634), *Francisella novicida* (ZP_03246790), and *Aquifex aeolicus* (NP_213072). The alignment was generated with CLUSTALW (http://www.ebi.ac.uk/Tools/msa/clustalw2/). A more comprehensive sequence alignment is provided in Supp. Fig. 1. A model of the predicted secondary structure of *E. coli* Hfq is presented below the conserved Hfq-sequence core. The cylinder represents an α-helix and the arrows β-sheets. This model was generated by means of the JPRED3 server (http://www.compbio.dundee.ac.uk/www-jpred/). (B) Sequence logo of the bacterial Sm1 motif. (C) Sequence logo of the eukaryotic LSm domain (adapted from http://pfam.sanger.ac.uk/family?acc=PF01423#tabview=tab0). (D) Sequence logo of the bacterial Sm2 motif. The Sm1 and Sm2 logos were generated by means of the MEME suite (http://meme.sdsc.edu/meme4_5_0/intro.html) based on the multiple alignment presented in panel A and the default settings of the server. (See colour version of this figure online at www.informahealthcare.com/mby)

a preference for RNA molecules that are rich in uridine, which seem to be accommodated around the pore in a constricted conformation that is stabilized by water molecules (Schumacher et al., 2002; Sauer and Weichenrieder, 2011). Two conserved residues exposed in the proximal face (F42 and H57 in *E. coli*) are relevant for selection of U-rich segments (Mikulecky et al., 2004; Sauer and Weichenrieder, 2011). As most sRNAs have typical Rho-independent terminators, which stretches usually contain a poly-U 3′-terminus (Wilson and von Hippel, 1995), Hfq may conceivably interact with the sRNA terminators and thus influence sRNA stability or turnover. Recent experimental evidence supports this hypothesis (Otaka et al., 2011). By contrast, the *E. coli* Hfq distal face has a preference for A-rich RNA oligos that bind in a circular conformation over the surface—as "much like a crown over the head of the monarch," a textual quotation of the smart analogy made by the authors (Link et al., 2009). This face would seem to contain a tripartite (A-R-E) RNA-binding motif with the 5′-adenosine binding to Site A (specific for

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

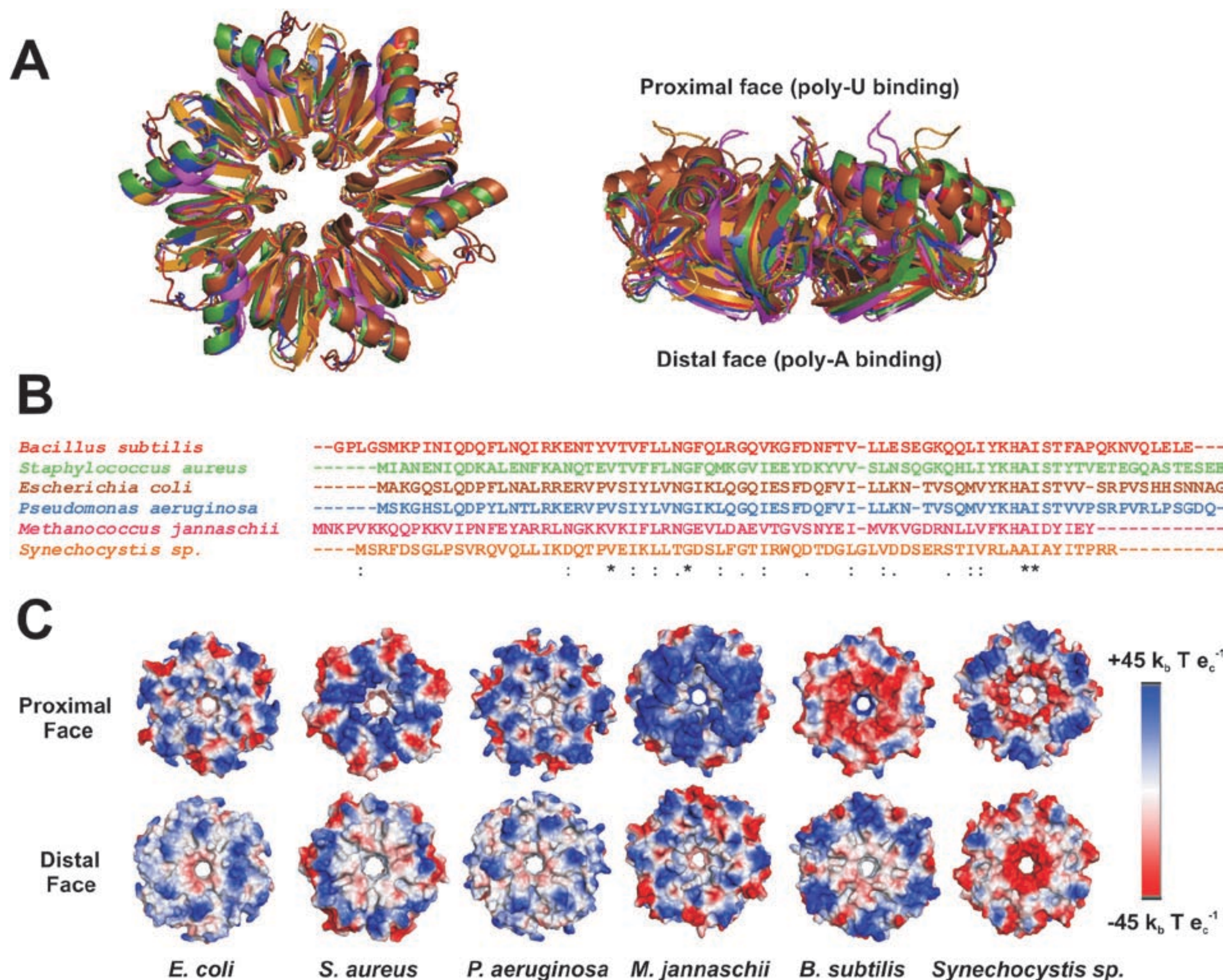


Figure 4. Three-dimensional structure conservation and surface-charge properties of Hfq. (A) Structural alignment of six Hfq homologs: *Escherichia coli* (3QHS), *Staphylococcus aureus* (1K1Q), *Synechocystis* sp. (3HFO), *Pseudomonas aeruginosa* (1U1S), *Bacillus subtilis* (3AHU), and the archaeon *Methanococcus jannaschii* (2QTX). The figure was generated with PYMOL (http://www.pymol.org/). The model shows the Hfq proximal face (left) and its on-edge view (right). (B) CLUSTAL-W sequence alignment of the Hfq-core sequences used to generate panel A. (C) Surface electrostatic potential of the Hfq homologs as calculated with APBS (Adaptative Poisson–Boltzmann Solver (Baker et al., 2001) and visualized by means of PYMOL. (See colour version of this figure online at www.informahealthcare.com/mby)

adenosines), the following nucleotide binding to the R site (specific for purines), and the third nucleotide binding to a nondiscriminatory RNA entrance-or-exit (E) site (Link et al., 2009). Interestingly, the proximal face of *B. subtilis* Hfq showed a slightly more strict preference for sequence motifs containing AG repeats (Someya et al., 2011), suggesting the existence of species-specific RNA recognition patterns among phylogenetic groups. Thus, each Hfq ring is able to simultaneously bind two different RNA molecules or even a single molecule bridging both faces around the oligomer rim. If a sRNA binds on one face and a cognate target mRNA does so on the second face, this ternary complex will lead to productive RNA duplex formation (Adamson and Lim, 2011).

Beneath the exposed surface of the protein, subtle residue interactions in solvent-inaccessible regions are also very critical for Hfq stability and function (Moskaleva et al., 2010; Someya et al., 2011). In line with the strong conservation of the Sm-2 histidine (Figure 3), the crystal structure of two *P. aeruginosa* Hfq variants (H57A and H57T) and a *B. subtilis* H57A mutant confirmed the relevance to Hfq structure of this deeply buried residue. These variants are less stable, chemically and thermally, than the wild-type protein (Moskaleva et al., 2010; Someya et al., 2011). In addition to its role in poly-U binding (Sauer and Weichenrieder, 2011), the buried histidine promotes hydrogen bonding between two neighbor monomers (Moskaleva et al., 2010). Thus, solvent-inaccessible intermonomer hydrogen bonds are critical for the stabilization of the Hfq quaternary structure.

Finally, the pore of the Hfq ring deserves comment. The diameter of the Hfq pore, as deduced from crystalline structures, ranges between 8–12 Å (Brennan and Link, 2007), whereas the diameter of a single-stranded RNA molecule is roughly 10 Å. The *S. aureus* Hfq pore, however, when complexed to a U-rich hexanucleotide becomes expanded to 15 Å (Schumacher et al., 2002). Molecular dynamic simulations furthermore predicted an expansion of the central pore of the *S. aureus* Hfq to up to 20 Å under conditions of high ionic strength (Lazar

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.
RIGHTSLINK

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

et al., 2010). An RNA molecule traverses the central Hfq hole in the manner reported for LSm proteins would thus appear feasible (Zaric et al., 2005). Although suggested previously (Schumacher et al., 2002; Valentin-Hansen et al., 2004; Link et al., 2009), the translocation of RNA through the Hfq pore has never been tested experimentally. A biophysical approach comparable to the one taken to elucidate the movement of single-stranded RNA across bionanopores (Butler et al., 2006) may be required to support or rule out any given hypothesis. The resulting conclusions may then agree with the biochemical evidence regarding the RNAP–Hfq interaction that will be described later in this review.

The Hfq C-terminus

As mentioned above, the divergence between Hfq homologs is seen with respect to the C-terminus, which region can vary in length and sequence (Figure 3; Supp. Fig. 1). Moreover, there are a few bacterial Hfq proteins with an unusually large C-terminus, as is the case of *Acinetobacter baylyi* (Schilling and Gerischer, 2009), *Burkholderia cenocepacia* (Ramos et al., 2011), *Moraxella catarrhalis* (Attia et al., 2008), and *Psychrobacter* spp. (Ayala-del-Rio et al., 2010), whose size roughly doubles that of typical Hfq proteins. Even among the proteins with custom size, the high variability of the Hfq C-terminus hampers the identification of functional motifs with biological relevance. Nevertheless, there are experimental clues that claim for a biological function of the C-tail. In *E. coli*, the C-terminus stabilizes the Hfq hexamer *in vitro* (Arluison et al., 2004) and is a key element for the interaction with nucleic acids (Updegrove et al., 2010). It is unclear which additional functions the C-terminal region confers to the folded Hfq core.

The first *E. coli* Hfq structure could be solved only if the protein lacked the last 36 residues (Sauter et al., 2003). The possibility of a highly flexible C-terminal extension, with no defined structure, arose as a feasible explanation. In fact, the C-terminal tail of *E. coli*' Hfq has a highly flexible architecture, with no obvious elements of secondary structure, and extends laterally away from the distal face of the Hfq-protein core (Beich-Frandsen et al., 2011a; Beich-Frandsen et al., 2011b). This appearance resembles the typical features of intrinsically disordered proteins, which species usually gain a defined structure upon interaction with other partners (Tantos et al., 2011). Perhaps such a role of "docking and folding" may be the function of the extended C-terminal region of Hfq. If so, the questions arise as to whether this putative functioning is unique to the Hfq of *E. coli* or applies to other Hfq proteins with extended C-termini as well and whether or not this region might likewise be functioning as a scaffold for docking either non-Hfq proteins or additional RNA substrates.

Notwithstanding, the contribution of the C-terminal region to riboregulatory processes is controversial. A few years ago, the C-terminus of *E. coli* Hfq was reported to be necessary for RNA transactions (Vecerek et al., 2008). A shortened Hfq variant lacking the last 37 residues (denoted as Hfq65) proved to be defective in the autocontrol of *hfq* mRNA translation and in RNA annealing, as the mutant protein failed to promote RhyB-mediated repression of *sodB* mRNA and DsrA-mediated stimulation of *rpoS* mRNA, both *in vitro* and *in vivo* (Vecerek et al., 2008). These results were not caused by a reduced Hfq stability since the Hfq65-protein level measured by quantitative immunoblotting was similar to that of the wild-type Hfq. Furthermore, ectopic expression of short Hfq proteins, such as those from *S. aureus* and *B. subtilis*, behaved phenotypically like the *E. coli* Hfq65 variant. These results pointed to the C-terminus as being an RNA-interaction surface with specificity for mRNAs (Vecerek et al., 2008). Further evidence in this direction has been recently provided by fluorescence resonance energy transfer (FRET) studies showing the inability of two truncated Hfq variants, Hfq65 and Hfq75, to bind two complementary RNA substrates, whereas the longer version Hfq85 succeeded to promote RNA duplex formation and to activate *rpoS* expression *in vivo* (Beich-Frandsen et al., 2011a). Thus, these results suggest that the portion between *E. coli*' Hfq residues 75 and 85 is directly involved in RNA binding.

Conversely, Olsen and colleagues demonstrated that the C-terminal region of the *E. coli* Hfq played only a minor role, if any, in riboregulation (Olsen et al., 2010). The expression of shortened Hfq variants lacking the C-terminal region (at 69 or 72 residues of total length) could complement the positive regulation on *rpoS* and the silencing of *sodB* and *ybfM* mRNAs, as demonstrated by northern blots, with no significant changes in the cellular Hfq69- and Hfq72-protein levels compared with those of the full-length Hfq. Another independent contribution in this sense came from the relatively null or low impact of C-tail deletion in two Hfq variants, Hfq65 and Hfq75, in terms of binding to sRNAs, to the *rpoS* leader, and of *in-vitro* duplex formation (Updegrove and Wartell, 2011). The contrasting discrepancies between the results of different studies could be attributed to specific experimental setups, like the size or nature of the RNA substrates. The *rpoS* transcript and its translational activation is a clear-cut example. Hfq increased the rate of *rpoS*–DsrA duplex formation only 2-fold for a short 140-nt version of the *rpoS* mRNA leader (567-nt long) (Lease and Woodson, 2004). Such modest contribution of Hfq opposes the well-known genetic requirement of the RNA chaperone for *rpoS* translational activation by DsrA (Sledjeski et al., 2001). However, using the full-length *rpoS* leader results in a completely different picture with association rates being 20- to 50-fold higher in the presence of Hfq (Soper and Woodson, 2008). The reason of this effect is not yet clear, but it may be related to alternative folding of the different *rpoS* mRNA leaders used in the experiments.

An alternative way to explore the function of the Hfq C-tail is through classical *in vivo*–complementation assays. Several *hfq* homologs could complement certain phenotypes of the *E. coli hfq* null mutant (Sonnleitner

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

et al., 2002; Lybecker et al., 2010; Nielsen et al., 2010; Sousa et al., 2010; Sobrero and Valverde, 2011). Thus, in spite of the differences in the C-terminal region, the conserved core sequence of Hfq can still lead to complementation, at least for the phenotypes studied in those works. Moreover, an Hfq with an unusual C-terminal region could fully complement this *E. coli* null mutant (Attia et al., 2008; Schilling and Gerischer, 2009). These results suggest that with respect to the fitness of bacteria, the C-terminal region of *E. coli* Hfq could be dispensable. That the Hfq region beyond the core is involved in specific regulatory mechanisms not yet reported, however, cannot be discarded.

Hfq mechanism and its role in sRNA networks

From *in-vitro* data, Hfq has been postulated as an RNA-binding protein (Sittka et al., 2009) involved in the facilitation of sRNA–mRNA interactions (Moller et al., 2002; Kawamoto et al., 2006; Soper and Woodson, 2008). Although sRNA–mRNA duplexes could be formed even in the absence of Hfq, the presence of this protein significantly accelerated that RNA pairing (Kawamoto et al., 2006; Soper et al., 2010). Two main mechanisms have been proposed to explain the role of Hfq in facilitating sRNA–mRNA interactions. The first of the proposed mechanisms assumes simultaneous binding of an sRNA and its target mRNA to Hfq, implying formation of ternary sRNA–mRNA–Hfq complexes in which Hfq "passively" bridges the two RNA substrates, as early suggested by Mikulecky and colleagues (Mikulecky et al., 2004). A few papers provide concrete experimental evidence in this sense. For instance, the full-length *rpoS* mRNA leader formed stable complexes with DsrA and Hfq as revealed by electrophoretic mobility shift assays (Soper and Woodson, 2008). More recently and using the same experimental approach, it was shown that the *fhlA* mRNA and OxyS sRNA could simultaneously bind to Hfq, when pre-formed OxyS–Hfq complex was titrated with the *fhlA* mRNA (Salim and Feig, 2010). Moreover, the utilized long *fhlA* leader construct could only be displaced from Hfq by adding simultaneously small RNA competitors with preference for Hfq proximal and distal faces, such as DsrA and A_{18}, respectively. Thus, it was deduced that the mRNA substrate could contact Hfq on both sides. In addition, kinetic data strongly suggested that an Hfq-bound mRNA substrate like *fhlA* would lead to formation of a productive ternary complex with an incoming sRNA, only if enough complementarity exists between the mRNA and the sRNA (Salim and Feig, 2010). Whether the formation of ternary complexes is a requisite for mRNA–sRNA annealing for mRNAs other than *rpoS* and *fhlA*, it remains to be determined.

The second proposed mechanism takes into account the documented RNA-chaperoning activity of Hfq in the remodeling of the structure of interacting RNAs (Schuppli et al., 1997; Moll et al., 2003). RNAse footprinting of the *sodB* mRNA leader revealed significant changes in its cleavage pattern upon binding to Hfq, particularly in a region that is complementary to its regulator sRNA, RyhB; but interestingly, the secondary structure of the riboregulator was barely affected by Hfq binding (Geissmann and Touati, 2004). In this way, Hfq facilitates exposure of the sequence targeted by the sRNA leading to formation of a *sodB*–RyhB–Hfq ternary complex, from which the paired RNAs and Hfq could be released by exposure to competing ligands (Afonyushkin et al., 2005). A significant contribution of mRNA restructuring by Hfq has been recently demonstrated for the *rpoS*–DsrA interaction (Soper et al., 2011). With biochemical binding assays using mutant RNA substrates, the authors have shown that (1) the formation of stable ternary complexes between the long *rpoS* leader, DsrA sRNA and Hfq previously observed in the same lab (Soper and Woodson, 2008), do require RNA complementarity; 2) a major reason for the facilitation of DsrA annealing is the remodeling of the mRNA secondary structure (Soper et al., 2011). These results, however, do not totally exclude the possibility that the two proposed mechanisms for Hfq to bring an mRNA and its sRNA partner into contact, do contribute with varying degrees depending on the mRNA–sRNA pair (Arluison et al., 2007a).

Although Hfq is a key agent in the RNA transactions of riboregulation networks, a defined and generalized mechanistic model for Hfq action is not yet available. Little is known about how bacterial sRNAs are recognized and loaded onto the Hfq-protein scaffold during their interaction with the target mRNAs. In addition, it must be stressed that most of our knowledge on the role of Hfq in RNA transactions derives from detailed studies of sRNAs in *E. coli* and *Salmonella*. More studies on Hfq-dependent sRNA-based riboregulation are required in other phylogenetic groups to challenge the paradigms based on enterobacterial Hfq.

As to the stoichiometry of the ternary complexes between Hfq and the RNA substrates (Brennan and Link, 2007), little within the available information has changed; and this obscure aspect of Hfq mechanism has not yet been clarified. Hfq has been reported to bind to DsrA *in vitro* not only at a 1:1 (Mikulecky et al., 2004; Hwang et al., 2011) but also at a 2:1 ratio (Sun and Wartell, 2006; Wang et al., 2011). Similar results have been shown for Hfq and the *rpoS*–mRNA leader, at either a 1:1 (Mikulecky et al., 2004) or a 2:1 ratio (Lease and Woodson, 2004). A 1:1 stoichiometric ratio between Hfq and either the domain II of DsrA or an A_{18} RNA substrate has been recently reported (Updegrove et al., 2011). Mass spectrometry signals of cross-linked ternary complexes (Hfq-DsrA[DII]-A_{18}) are consistent with a 1:1:1 stoichiometry, although the complex appeared to be quite unstable (Updegrove et al., 2011). Similarly, single-molecule fluorescence techniques have recently illustrated how Hfq promotes unwinding and annealing of two different RNA substrates, providing an *in-vitro* real-time tracking of the chaperoning role of Hfq in the interaction between DsrA–*rpoS* (Hwang et al.,

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.
RIGHTSLINK

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

2011). In terms of stoichiometry, this work demonstrates that a single Hfq hexamer is sufficient to promote DsrA–*rpoS* interaction (Hwang et al., 2011). Concomitantly, new structural and biophysical data support a 2:1 stoichiometry between two *E. coli* Hfq hexamers and the U-rich internal segment of DsrA laying between stem-loops I and II (AU_6A; nucleotides 28–35) (Wang et al., 2011), in line with previous reports (Lease and Woodson, 2004). It was found in the crystal structure that AU_6A was bound to the proximal face of one Hfq hexamer, resembling AU_5G complexed to *S. aureus* Hfq (Schumacher et al., 2002), and surprisingly, that A28 docks within the purine selective site of the distal face of a second Hfq hexamer, as previously described for poly(A) (Link et al., 2009). The cooperation of two Hfq hexamers upon binding to full-length DsrA was confirmed in solution by nuclear magnetic resonance (NMR) spectroscopy and FRET analysis (Wang et al., 2011). Such cooperative inter-hexameric interaction occurring with a distal to proximal preference for DsrA binding determines the approach of two different Hfq hexamers and may thus facilitate the exposure of DsrA sequence required for the annealing with its target mRNA *rpoS*. As most of the AU_6A nucleotides base pair with *rpoS*, it is expected that the formation of the sRNA–mRNA duplex would cause partial dissociation of the sRNA from Hfq, allowing protein recycling (Wang et al., 2011).

The obvious discrepancies in the stoichiometry of complexes formed by Hfq and RNA ligands like DsrA could result, again, from the different biophysical methods used or from differences in either the preparations of recombinant Hfq or the RNA substrates involved, as highlighted in the previous section. Wang and colleagues have crystallized a complex between a C-terminal truncated version of Hfq (Hfq65) and AU_6A and confirmed the Hfq:DsrA 2:1 ratio by NMR and FRET using the same short Hfq version (Wang et al., 2011); Hwang and colleagues, instead, have utilized a full-length *E. coli* Hfq protein and a different portion of the DsrA molecule (HWang et al., 2011). Clearly, unifying studies are mandatory to conclude about the stoichiometry of a particular Hfq–sRNA–mRNA interaction.

In any case, an Hfq-chaperoning mechanism based on *in-vitro* data must be considered with care, as any hypothesis could be far removed from what really occurs *in vivo*. The Hfq protein forms extremely stable complexes with RNA substrates *in vitro*, but the corresponding dissociation rates are so low that they cannot explain the function of this protein as a facilitator of sRNA–mRNA interactions over a biologically compatible time scale. Several sRNAs have been shown recently to be able to bind to Hfq with similar affinities *in vitro* under the same experimental conditions (Olejniczak, 2011). In that work, the sRNAs were chosen on the basis of their dissimilar sequence and secondary structures, but they all exhibited comparable association rate constants. This observation implies that the limiting step for the protein–RNA interaction is the diffusion of the sRNA to Hfq. Once formed, though, the different sRNA–Hfq complexes showed very low dissociation rates (Olejniczak 2011), confirming previous data on the great stability of Hfq–sRNA complexes *in vitro* (Arluison et al., 2004; Geissmann and Touati, 2004; Mikulecky et al., 2004; Soper and Woodson, 2008; Holmqvist et al., 2010). The dissociation rates of all the sRNAs studied increased in the presence of a higher concentration of competitor sRNA, but with different competitive performances, this may reflect the ability of each sRNA to actively displace the ones bound to Hfq (Olejniczak, 2011).

Within the same line of evidence, another recent publication succeeded in solving the "strong-binding–high-turnover paradox." Fender and colleagues demonstrated that the presence of sRNA competitors increased the dissociation rates of Hfq complexes, favoring less stable complexed ribonucleoproteins (Fender et al., 2010). The half-lives of the complexes measured in this research are compatible to the time scale observed *in vivo* for sRNA-mediated gene regulation (Masse et al., 2003). This correspondence suggests a dynamic model of RNA cycling on Hfq, in which scheme the RNA bound to Hfq can be actively displaced by a competitor RNA (Mikulecky et al., 2004; Link et al., 2009; Moon and Gottesman, 2011; Wang et al., 2011). Within this scenario, a given RNA may be considered to form different "productive" complexes with Hfq, or intermediate states, in which the RNA displays different affinities; much like what occurs with substrates that display alternative and nonproductive binding to an enzyme active site. Under such a hypothesis, the displacement of the RNA substrates on Hfq should be an RNA-concentration–driven process. Such sRNA-mediated displacement in Hfq-sRNA complexes can have a huge impact on riboregulation networks *in vivo* (Hussein and Lim, 2011; Moon and Gottesman, 2011). Interestingly, a similar conclusion was driven from a mathematical framework to model Hfq-dependent sRNA regulatory networks (Adamson and Lim, 2011). The model firstly considered "isolated" sRNA–mRNA interactions with separate binding sites for mRNA and sRNAs on the Hfq hexamer leading to formation of cognate mRNA–sRNA duplexes. In this context, the most effective duplex formation rate and the range of Hfq concentrations that results in maximal duplex formation ("robustness") were favored if both mRNA and sRNA bind and dissociate cooperatively to Hfq. The model next incorporated a feature of the real sRNA network in the cell environment, which is the multiplicity of Hfq ternary complexes, either cognate leading to duplex formation or noncognate unproductive ones. In this scenario, the association kinetics and abundance of competing RNAs become strongly influential in duplex formation of a certain sRNA–mRNA pair. It was predicted that imbalances in sRNA or mRNA production could alter Hfq function and effective duplex formation. Thus, such general and basic mathematical model (Adamson and Lim, 2011) succeeded to explain experimental findings from *in-vivo* data indicating that Hfq may be a limiting entity for sRNA functioning and

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

that overexpression of a given sRNA or target mRNA without its cognate partner can affect the regulatory activity of an unrelated sRNA (Hussein and Lim, 2011; Moon and Gottesman, 2011). Therefore, the expression of a sRNA and its mRNA target should be kept within a certain range of levels in order to avoid conflicts within the riboregulatory network as a result of Hfq depletion. Accordingly, care must be taken when interpreting the results of sRNA overexpression on the regulation of target genes.

All this complementary information permits a more realistic view of the role of Hfq in the RNA transactions that occur during sRNA-mediated gene regulation. Further experimental evidence will be required, however, to understand the dynamics of this process in the bacterial cell milieu. From the models proposed in recent publications (Fender et al., 2010; Adamson and Lim, 2011; Hopkins et al., 2011; Hussein and Lim, 2011; Moon and Gottesman, 2011) and the interesting conclusion drawn by Vogel and Luisi in a contemporary review (Vogel and Luisi, 2011), a new view of Hfq-mediated riboregulation at the molecular level has emerged. The pool of free Hfq inside the cell should be very low, given the abundance of possible RNA or DNA substrates and the high Hfq-binding affinities. Therefore, as most of the Hfq is bound to a nucleic acid, a newly synthesized sRNA that is induced by a given stimulus has to be exchanged with the bound RNA in order to produce the regulatory effect. The active cycling of RNA would be necessary in order to coordinate the half-life of the ribonucleoprotein complexes with the time scale for riboregulation. Thus, in order to obtain a specific displacement of a competitor RNA by a given sRNA, the competition must possess a certain degree of specificity. Coexpression of an sRNA with its corresponding mRNA could favor this competition; but, in addition, chromatin rearrangements could lead to the formation of specific "riboregulosomes," where sRNA and mRNA expression becomes coordinated not only in time but also in space (Vogel and Luisi, 2011). We could imagine that the Hfq pool provides a discontinuous "contact surface" to promote the sRNA–mRNA interaction, much in the way that the surface of an inorganic catalyst such as platinum or zeolite provides a contact interface for diffusing molecules. Furthermore, Hfq could even have a more "active" regulatory role because this protein can disrupt secondary structures (Arluison et al., 2007a) and can function as well as an ATPase (Sukhodolets and Garges, 2003, Arluison et al., 2007b), perhaps to compensate for the decline in entropy associated with the formation of large ribonucleoprotein complexes. Alternatively, Hfq may actively recruit other protein factors for participation in the "riboregulosome."

Hfq as a DNA-binding protein

Substantial experimental evidence has been provided in support of the view that Hfq is a RNA-binding protein, but the broad Hfq specificity for sRNAs and mRNAs would even seem to extend to other types of nucleic acids as well. For instance, Hfq can bind DNA (Takada et al., 1997; Updegrove et al., 2010; Geinguenaud et al., 2011) and other cellular nucleic acids, such as tRNAs (Lee and Feig, 2008). In fact, Hfq was identified as one of the 12 most abundant proteins in the *E. coli* nucleoid, representing about 24% of the total proteins associated to genomic DNA in exponential growth phase (Talukder et al., 2006). This abundance in the nucleoid is comparable to that of Fis and HU proteins (Talukder et al., 2006). Among over 200 proteins identified by a proteomic analysis of *E. coli* nucleoids, Hfq was almost equally distributed in the nucleoid and in the cytoplasmatic fraction (Ohniwa et al., 2011), matching early reports on the subcellular localization of *E. coli*' Hfq (Kajitani et al., 1994). It is not clear whether this *in-vivo* association of Hfq to the nucleoid is to the DNA or to the RNA fraction, or both.

The DNA-binding ability of Hfq has been studied *in vitro* using purified molecules. Geinguenaud and collaborators combined vibrational spectroscopy and neutron scattering to show that Hfq induces a partial opening of DNA sequences enriched in adenine and thymine upon interaction with deoxyadenosine residues. Such a target preference with respect to DNA substrates coincides with that feature of typical promoter sequences. DNA binding would appear to have a certain structural requirement since Hfq was found to have copurified with DNA fragments that had higher helical axis curvatures than the average for random sequences of the *E. coli* genome (Updegrove et al., 2010), confirming a pioneer report on the higher affinity of Hfq for curved DNA rather than for noncurved DNA (Azam and Ishihama, 1999).

Both the distal face and the C-terminus of Hfq are implicated in the interaction with DNA (Updegrove et al., 2010). The deletion of the 37 amino acids in the C-terminus or the mutations Y25A and K31A completely abolished the interaction with DNA (Updegrove et al., 2010). As these two point mutations have the same effect as the deletion of the C-terminal region, we can argue that the binding of DNA to these two separate sites is not independent. Thus, there must be a connection between them: DNA could interact and move through Hfq using both surfaces. Maybe the C-terminal region, which is the most exposed part of Hfq, serves to anchor the hexamer to the DNA molecule and the residues Y25 and K31 helped in the formation of specific hydrogen bonds necessary for the interaction. Interestingly, mutation in R16A, which is situated in the edge of the protein, also abolished the interaction, meanwhile R17A mutation slightly altered the complex formation (Updegrove et al., 2010). Thus, to some extent, the edge of Hfq is also involved in the interaction with DNA. The sequence of the Hfq-associated DNA indicated that the protein had bound preferentially to gene segments encoding membrane proteins (65% of all identified sequences), an extraordinary finding for a form of nonspecific binding (Updegrove et al., 2010). Moreover, a common DNA motif was found in those

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

associated sequences, (A/T)T(A/G)TGCCG, which consensus is clearly different from the deoxyadenosine-rich sequences described by Guingenaud and colleagues. Neither of the genes identified encodes mRNAs that are subject to Hfq-dependent riboregulation, nor do they flank intergenic regions encoding sRNAs. These observations highlight some differences in the *in-vitro* and *in-vivo* specificity of Hfq. When the *E. coli* genome was inspected for the occurrence of the (A/T)T(A/G) TGCCG motif (Search pattern routine in the COLIBRI web server; http://genolist.pasteur.fr/Colibri/), 924 perfect hits were identified. If only one hexamer were bound per motif, about 15% of the cellular Hfq pool (924 out of 5800 hexamers) would be directly associated with the *E. coli* nucleoid. This figure correlates with the reported fraction of Hfq molecules (10–20%) associated with the chromosome (Kajitani et al., 1994).

An intriguing finding is that among the 12 most abundant proteins in the *E. coli* nucleoid (Azam and Ishihama, 1999), Hfq copurified only with the histone-like protein HU upon nuclease treatment (Butland et al., 2005) (Supp. Table 3). HU has been considered the bacterial counterpart of eukaryotic histones, with an important role in nucleoid architecture and transcriptional activity (Pontiggia et al., 1993; Kar et al., 2005). But HU is rather more analogous in function to the eukaryotic HMG proteins that recruit transcription factors to enhancer regions or to the RNAP (Verrier et al., 1997; Najima et al., 2005; Berger et al., 2010). Thus, HU may also recruit Hfq to the nucleoid and promote its interaction with specific DNA sequences (Tolstorukov et al., 2005).

Thus, considering the demonstrated DNA binding activity of Hfq and the DNA sequence and structure constrains for such binding, as well as the significant proportion of Hfq that is found in the nucleoid, it may be hypothesized that Hfq has an additional role in the nucleoid related to DNA topology and transcription, on top of the proposed "riboregulosomes." Unfortunately, the *in-vivo* experimental evidence for such hypotheses—though they are appealing (Figure 1)—is still inconclusive. At least *in vitro*, Hfq was shown to have a stimulatory effect on the transcription yield from a supercoiled plasmid template at high protein concentration (*ca.* 1 μM of hexamer) (Sukhodolets and Garges, 2003). In addition, deeper insights into the dynamics of Hfq in the nucleoid are required.

Hfq-protein complexes and the impact on RNA metabolism

Thus far, we have considered the interactions of Hfq only with nucleic acids, and particularly with RNAs. Hfq, though, displays a very interesting property that may have an impact on riboregulatory mechanisms as well as on other central processes of gene expression: the interaction with other proteins (Figure 1). In fact, experiments have verified that Hfq interacts with at least 30 proteins or large protein complexes in *E. coli* (Supp. Table 3) (Butland et al., 2005). Remarkably, most of the proteins binding to Hfq are involved in major steps of the flow of genetic information: transcription, translation, RNA metabolism, and protein folding (Supp. Table 3). In most instances, whether these interactions are direct (e.g., involving Hfq-protein physical contacts) or indirect via an RNA or DNA molecule remains to be elucidated. The latter possibility seems to be the circumstance for at least CspC (Cohen-Or et al., 2010) and CsrA (Sorger-Domenigg et al., 2007). A more complex interaction scenario would be necessary to explain the odd finding of the cytoplasmic *E. coli* protein LpxD (involved in the LPS lipid-A synthesis) among the group of confirmed Hfq protein partners (Supp. Table 3). The *lpxD* mRNA was reported to be subject to post-transcriptional regulation by Hfq (Guisbert et al., 2007) and the *lpxD* mRNA was seen to coimmunoprecipitate with Hfq (Sittka et al., 2009). We might speculate that if *lpxD* mRNA translation and polypeptide elongation were arrested by an sRNA–Hfq–dependent mechanism, the ribosomes would then simultaneously hold the *lpxD* mRNA, Hfq, and a portion of the LpxD nascent polypeptide. In line with this notion, all species have been bound by Hfq *in vitro* (Butland et al., 2005; Sittka et al., 2009). As yet, however, no *in-vivo* direct experimental evidence exists for such higher-order functional complexes.

The best studied example of a direct interaction between Hfq and a protein partner is with RNAse E in the RNA degradosome, a multiprotein complex responsible for RNA degradation (Aiba, 2007). RNAse E is the catalytic endoribonuclease component of the RNA degradosome, which molecular complex also includes polynucleotide phosphorylase, RNA helicase B, and enolase (Carpousis, 2007). The C-terminal scaffold region of RNAse E is critical for the binding to Hfq (Morita et al., 2005). Hfq makes contact with the segment spanning residues 702–750 of RNAse E (Ikeda et al., 2011), but the portion of Hfq that is interacting with that stretch of the RNAse E still remains obscure. Nonetheless, sRNA-dependent silencing could still be achieved for *ptsG* and *sodB* mRNAs in an *rne* mutant (Morita et al., 2006). The role of the Hfq–RNAse E association in the degradosome would thus appear to couple mRNA translational repression with irreversible degradation. The Hfq–RNAse E interaction seems to be highly conserved in bacteria. The following observations support this hypothesis: (1) Hfq–RNAse E complexes have been isolated from *R. leguminosarum* cells (Zhang and Hong, 2009); (2) MicX sRNA from *Vibrio cholerae* is processed by RNAse E in a Hfq-dependent fashion (Davis and Waldor, 2007); (3) the stability of certain sRNAs of *S. typhimurium*, such as SrlA or MicA, depends on this complex (Viegas et al., 2007).

The ribosomal protein S1 and RNAP seem to be directly associated with Hfq as well. Different immobilized *E. coli* sRNAs (DsrA, MicF, OxyS, RyhB, or Spot42) were able to precipitate Hfq, along with S1 protein and the RNAP β subunit, from *E. coli* cell extracts (Windbichler et al., 2008). Consistent with this notion, ribosomal protein S1

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

and Hfq were present at a stoichiometric ratio in preparations of RNAP from *E. coli* cell cultures (Sukhodolets and Garges, 2003). *In-vitro* recombinant Hfq shows little or no affinity for the RNAP core region but interacts with the RNAP-S1 complex (Sukhodolets and Garges, 2003). Similarly, purified recombinant Hfq and S1 protein failed to interact *in vitro* (Vecerek et al., 2010), raising the possibility that the observed interactions were indirect via binding of both proteins to an RNA fragment. However, pull down experiments using Hfq as a bait and *E. coli* cell extracts treated with a promiscuous nuclease, revealed the presence of S1 and RNAP subunits among the Hfq-captured preys (Supp. Table 3) (Butland et al., 2005). Such findings argue in favor of a higher order complex formation involving at least Hfq, S1 protein, and RNAP and raise the question as to the biological significance of this complex—whether it is involved in riboregulation or is independent of sRNAs, and whether Hfq could be positioned at the RNA exit channel of the RNAP available for binding to appropriate substrates. As an RNA chaperone, Hfq can promote the appropriate mRNA folding either for a more efficient translation or to attenuate transcriptional pausing (Le Derout et al., 2010). Moreover, RNA remodeling may be assisted by the aforementioned Hfq ATPase activity (Sukhodolets and Garges, 2003; Arluison et al., 2007b), thus suggesting a role for Hfq in transcriptional-translational–coupling mechanisms (Figure 1).

Hfq may interact with two other proteins involved in RNA metabolism, poly(A) polymerase I and the exoribonuclease polynucleotide phosphorylase (Mohanty et al., 2004). These enzymes are directly involved in the regulation of mRNA turnover (Santos et al., 2006; Viegas et al., 2007). The interaction of Hfq with the 3′-terminus of an RNA substrate induces its polyadenylation through poly(A) polymerase I and the consequent degradation of the RNA molecule (Folichon et al., 2005). Another consequential Hfq partner is the essential motor protein Rho. This complex was first identified *in vivo* (Butland et al., 2005), and the identification was recently confirmed in *E. coli* both *in vitro* and *in vivo* (Rabhi et al., 2011). Hfq forms a reasonably stable complex with Rho, so as to promote transcription antitermination *in vitro* because upon Hfq binding, the ATPase and helicase activity of Rho are blocked (Rabhi et al., 2011). The distal face of Hfq would moreover appear to be involved in antitermination. Thus, the ability of Hfq to interact directly with RNAP and with Rho positions this protein at a key regulatory position in cellular transcriptional activity (Figure 1).

The question of the autonomy of Hfq action

The role of Hfq has been explored in many bacterial models, from Gram-negative to Gram-positive bacteria, from human pathogens to beneficial species in plants (Table 1). In general, the absence of Hfq results in pleiotropic effects on the physiology of bacteria, including reduced growth and reduced tolerance to stress conditions (Table 1). Thus, the regulation of gene expression by Hfq, through interactions with sRNAs, plays a central role in the fitness of bacteria. Such a multiple role would seem extremely demanding for a single protein and would thus beg the question as to whether alternative genetic recourses also exist as well as whether cells complement the function of Hfq with other companion chaperones.

In this regard, a recent paper shows that a small (155 residue) and highly conserved hypothetical protein, designated as YbeY in *E. coli*, is involved in riboregulation in *S. meliloti* strain 1021 (Pandey et al., 2011). YbeY and the *S. meliloti* homolog (SMc01113) show a high degree of amino-acid–sequence conservation. Sequence- and structural-homology searches suggest that the YbeY proteins resemble the MID domain of the Argonaute proteins, fundamental components of the eukaryotic RNA-induced silencing complex (Czech and Hannon, 2011). An SMc01113 mutant reproduced several phenotypes of the *S. meliloti hfq* mutant and was found to have the lower intracellular sRNA levels of the *hfq*-minus strain (Pandey et al., 2011). No physical interaction, however, was evidenced between the YbeY homolog and Hfq in a two-hybrid assay. Since both mutants exhibited similar phenotypes, both proteins could not have been acting independently in the same regulatory pathways because the absence of one would have been complemented by the presence of the other. Whether YbeY influences *hfq* expression or function, however, remains to be determined.

In *E. coli*, the 222–amino-acid basic protein ProQ emerged as another RNA chaperone (Chaulk et al., 2011). This protein has been proposed to control the expression of *proP*, which encodes a symporter involved in the osmo-adaptative response (Keates et al., 2010). The C-terminal domain of this protein exhibited a significant structural similarity to Hfq, despite the limited sequence conservation between the two proteins. None of the characteristic Sm-1 or Sm-2 Hfq motifs were detected in ProQ. Nevertheless, the *in-silico* structural model of the ProQ C-terminal domain resembled the analogous Hfq fold (Chaulk et al., 2011). The Hfq-like domain of ProQ could operate as an RNA chaperone to promote RNA-strand exchange and the RNA–RNA–annealing reaction. Nevertheless, the ProQ molecular mechanism and its possible RNA substrates remain unknown. A molecular dissection of ProQ structure will be required. The N-terminal domain of ProQ is similar to FinO, another RNA-binding protein (Arthur et al., 2011). A tempting speculation would be that the Hfq-like C-terminal domain of ProQ can assist in RNA exchange and that the FinO-like domain confers specific RNA-binding properties to the protein. An investigation of whether this protein has a role in bacterial physiology other than the control of *proP* expression, and if so, whether ProQ, like Hfq, oligomerizes *in vivo* to create RNA-binding sites would indeed seem relevant.

In general, only one *hfq* gene is present per genome complement. As expected, exceptions to this rule exist.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

Members of the *Burkholderia cepacia* complex encode two distinct and functional Hfq-like proteins (Ramos et al., 2011). One is a typical bacterial Hfq protein and is essential for the virulence of these bacteria (Sousa et al., 2010); the other is unusually long (188 amino acids), is active in RNA and DNA binding, and forms trimers *in vitro* (Ramos et al., 2011). This unusual gene duplication seems to be restricted to strains of the *B. cepacia* complex. A strain mutated in Hfq2 is not able to cope with environmental stresses and is not virulent, despite the presence of Hfq1 (Ramos et al., 2011). The *hfq* and *hfq2* genes would seem to have an inverse temporal-expression pattern since the *hfq* mRNA accumulates during the exponential phase, whereas the *hfq2* mRNA reaches maximum levels during the stationary phase. These observations suggest that both Hfq proteins are required for an optimal physiology and riboregulation in *B. cenocepacia*.

Biotechnological implications of an understanding of Hfq properties

The growing bulk of experimental data collected on the properties of the RNA chaperone Hfq and its role in bacterial well-being may lead to innovative practical applications. In the eukaryotes, small-interfering RNAs emerged as an invaluable tool for reverse genetics. A similar silencing strategy could conceivably be achieved in bacteria by using artificial sRNAs for the stringent control of gene expression based on target-mRNA base-pairing and coupled translation inhibition and/or mRNA degradation—all of these effects through the participation of Hfq. Moreover, the mRNA-target multiplicity of certain sRNAs could be exploited to globally shut down key physiologic processes (Guillier and Gottesman, 2008; Balbontin et al., 2010; Nielsen et al., 2010; Papenfort et al., 2010). The minimal modules of an artificial sRNA (atsRNA) gene would be: (1) a base-pairing region targeting an mRNA leader, (2) an Hfq-binding site, and (3) a Rho-independent terminator. The atsRNA should be under the control of an appropriate inducible promoter. The Hfq-binding site could be derived from characterized sRNAs and even further engineered to enhance the efficiency of the first repressing module, as has been recently demonstrated for the RhyB–*sodB* interaction (Hao et al., 2011). The rational design of atsRNAs was experimentally addressed in *E. coli* in order to silence the essential genes *murA* (encoding UDP-N-acetylglucosamine-enolpyruvoyl transferase), *trmA* (encoding tRNA [m^5-U54] methyltransferase), and *ygjD* (encoding a putative O-sialoglycoprotein endopeptidase) (Man et al., 2011). Induction of atsRNA expression inhibited the growth of the *E. coli* strain MC4100 at 30°C. The inhibition correlated with a direct mRNA silencing mediated by translational repression through the assistance of Hfq and RNAse E (Man et al., 2011). Similar atsRNA constructs, though, failed to silence the α-hemolysin mRNA in the Gram-positive *S. aureus*, thus raising the possibility that riboregulation may require specific additional factors in Gram-positive bacteria.

As many bacterial pathogens display avirulent phenotypes in different host models upon *hfq* knockout (Table 1), the attenuated mutant strains could be used as vaccine components. This outcome was documented with *S. typhimurium* (Sittka et al., 2007): A single oral immunization dose with a *S. typhimurium hfq*-deletion mutant conferred protection on mice against a subsequent oral challenge of the virulent wild-type strain (Allam et al., 2011). The antigens derived from the mutant strain were somehow more effectively presented by dendritic cells to T-cells than were the wild type, thus contributing to the protective immunity seen with the *hfq* mutant (Allam et al., 2011). Another such example is with respect to *Vibrio alginolyticus*, the causative agent of fish vibriosis in several commercial species, which disease represents a concern to the mariculture industry. As reported for *S. typhimurium*, either a single injected dose or immersion in a suspension of a *V. alginolyticus hfq*-mutant cells conferred significant levels of protection on zebra fish against a subsequent challenge with the virulent wild-type strain (Liu et al., 2011). These studies suggest that *hfq*-mutant derivatives could be considered and tested as novel live oral-vaccine candidates against bacterial infectious diseases.

Conclusion

Nearly 45 years after its discovery as a specific factor for the replication of a coliphage (Franze de Fernandez et al., 1968), the bacterial protein Hfq is recognized today as an RNA-binding protein promoting riboregulatory mechanisms (Figure 1). The *hfq* mutations are associated with broadly pleiotropic phenotypes, which reflect the loss of fine tuning-regulatory mechanisms of gene expression is therefore hardly surprising (Table 1). Much of the recent effort summarized above has focused on the biochemical and functional characterization of Hfq on the basis of its preference for RNA. Nevertheless, the molecular details of sRNA and mRNA discrimination and its chaperoning activity are still far from being clearly elucidated. The understanding of the role of Hfq at the molecular level may benefit from a deeper investigation of its subcellular location and distribution as well as from a closer analysis of its ability to interact with other macromolecules. The possibility that Hfq participates in higher-order macromolecular and functional complexes is an extraordinary topic that merits further attention.

Declaration of interest

The authors report no conflicts of interest.

References

Adamson DN, Lim HN. (2011). Essential requirements for robust signaling in Hfq dependent small RNA networks. PLoS Comput Biol, 7, e1002138.

Afonyushkin T, Vecerek B, Moll I, Bläsi U, Kaberdin VR. (2005). Both RNase E and RNase III control the stability of sodB mRNA upon

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

translational inhibition by the small regulatory RNA RyhB. Nucleic Acids Res, 33, 1678–1689.

Aiba H. (2007). Mechanism of RNA silencing by Hfq-binding small RNAs. Curr Opin Microbiol, 10, 134–139.

Ali Azam T, Iwata A, Nishimura A, Ueda S, Ishihama A. (1999). Growth phase-dependent variation in protein composition of the Escherichia coli nucleoid. J Bacteriol, 181, 6361–6370.

Allam US, Krishna MG, Lahiri A, Joy O, Chakravortty D. (2011). Salmonella enterica serovar Typhimurium lacking hfq gene confers protective immunity against murine typhoid. PLoS ONE, 6, e16667.

Arluison V, Derreumaux P, Allemand F, Folichon M, Hajnsdorf E, Régnier P. (2002). Structural Modelling of the Sm-like Protein Hfq from Escherichia coli. J Mol Biol, 320, 705–712.

Arluison V, Folichon M, Marco S, Derreumaux P, Pellegrini O, Seguin J, Hajnsdorf E, Regnier P. (2004). The C-terminal domain of Escherichia coli Hfq increases the stability of the hexamer. Eur J Biochem, 271, 1258–1265.

Arluison V, Hohng S, Roy R, Pellegrini O, Régnier P, Ha T. (2007a). Spectroscopic observation of RNA chaperone activities of Hfq in post-transcriptional regulation by a small non-coding RNA. Nucleic Acids Res, 35, 999–1006.

Arluison V, Mutyam SK, Mura C, Marco S, Sukhodolets MV. (2007b). Sm-like protein Hfq: location of the ATP-binding site and the effect of ATP on Hfq– RNA complexes. Protein Sci, 16, 1830–1841.

Arnvig KB, Young DB. (2009). Identification of small RNAs in Mycobacterium tuberculosis. Mol Microbiol, 73, 397–408.

Arthur DC, Edwards RA, Tsutakawa S, Tainer JA, Frost LS, Glover JN. (2011). Mapping interactions between the RNA chaperone FinO and its RNA targets. Nucleic Acids Res, 39, 4450–4463.

Attia AS, Sedillo JL, Wang W, Liu W, Brautigam CA, Winkler W, Hansen EJ. (2008). Moraxella catarrhalis expresses an unusual Hfq protein. Infect Immun, 76, 2520–2530.

Ayala-del-Río HL, Chain PS, Grzymski JJ, Ponder MA, Ivanova N, Bergholz PW, Di Bartolo G, Hauser L, Land M, Bakermans C, Rodrigues D, Klappenbach J, Zarka D, Larimer F, Richardson P, Murray A, Thomashow M, Tiedje JM. (2010). The genome sequence of Psychrobacter arcticus 273-4, a psychroactive Siberian permafrost bacterium, reveals mechanisms for adaptation to low-temperature growth. Appl Environ Microbiol, 76, 2304–2312.

Azam TA, Ishihama A. (1999). Twelve species of the nucleoid-associated protein from Escherichia coli. Sequence recognition specificity and DNA binding affinity. J Biol Chem, 274, 33105–33113.

Baba S, Someya T, Kawai G, Nakamura K, Kumasaka T. (2010). Expression, crystallization and preliminary crystallographic analysis of RNA-binding protein Hfq (YmaH) from Bacillus subtilis in complex with an RNA aptamer. Acta Crystallogr Sect F Struct Biol Cryst Commun, 66, 563–566.

Babitzke P, Baker CS, Romeo T. (2009). Regulation of translation initiation by RNA binding proteins. Annu Rev Microbiol, 63, 27–44.

Bai G, Golubov A, Smith EA, McDonough KA. (2010). The importance of the small RNA chaperone Hfq for growth of epidemic Yersinia pestis, but not Yersinia pseudotuberculosis, with implications for plague biology. J Bacteriol, 192, 4239–4245.

Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. (2001). Electrostatics of nanosystems: application to microtubules and the ribosome. Proc Natl Acad Sci USA, 98, 10037–10041.

Balbontín R, Fiorini F, Figueroa-Bossi N, Casadesús J, Bossi L. (2010). Recognition of heptameric seed sequence underlies multi-target regulation by RybB small RNA in Salmonella enterica. Mol Microbiol, 78, 380–394.

Barra-Bily L, Fontenelle C, Jan G, Flechard M, Trautwetter A, Pandey SP, Walker GC, Blanco C. (2010a). Proteomic alterations explain phenotypic changes in Sinorhizobium meliloti lacking the RNA chaperone Hfq. J Bacteriol, 192, 1719–1729.

Barra-Bily L, Pandey SP, Trautwetter A, Blanco C, Walker GC. (2010b). The Sinorhizobium meliloti RNA chaperone Hfq mediates symbiosis of S. meliloti and alfalfa. J Bacteriol, 192, 1710–1718.

Beich-Frandsen M, Vecerek B, Konarev PV, Sjöblom B, Kloiber K, Hämmerle H, Rajkowitsch L, Miles AJ, Kontaxis G, Wallace BA, Svergun DI, Konrat R, Bläsi U, Djinovic-Carugo K. (2011a). Structural insights into the dynamics and function of the C-terminus of the E. coli RNA chaperone Hfq. Nucleic Acids Res, 39, 4900–4915.

Beich-Frandsen M, Vecerek B, Sjöblom B, Bläsi U, Djinovic-Carugo K. (2011b). Structural analysis of full-length Hfq from Escherichia coli. Acta Crystallogr Sect F Struct Biol Cryst Commun, 67, 536–540.

Beisel CL, Storz G. (2010). Base pairing small RNAs and their roles in global regulatory networks. FEMS Microbiol Rev, 34, 866–882.

Berger M, Farcas A, Geertz M, Zhelyazkova P, Brix K, Travers A, Muskhelishvili G. (2010). Coordination of genomic structure and transcription by the main bacterial nucleoid-associated protein HU. EMBO Rep, 11, 59–64.

Berghoff BA, Glaeser J, Sharma CM, Zobawa M, Lottspeich F, Vogel J, Klug G. (2011). Contribution of Hfq to photooxidative stress resistance and global regulation in Rhodobacter sphaeroides. Mol Microbiol, 80, 1479–1495.

Bøggild A, Overgaard M, Valentin-Hansen P, Brodersen DE. (2009). Cyanobacteria contain a structural homologue of the Hfq protein with altered RNA-binding properties. FEBS J, 276, 3904–3915.

Bohn C, Rigoulay C, Bouloc P. (2007). No detectable effect of RNA-binding protein Hfq absence in Staphylococcus aureus. BMC Microbiol, 7, 10.

Brennan RG, Link TM. (2007). Hfq structure, function and ligand binding. Curr Opin Microbiol, 10, 125–133.

Butland G, Peregrín-Alvarez JM, Li J, Yang W, Yang X, Canadien V, Starostine A, Richards D, Beattie B, Krogan N, Davey M, Parkinson J, Greenblatt J, Emili A. (2005). Interaction network containing conserved and essential protein complexes in Escherichia coli. Nature, 433, 531–537.

Butler TZ, Gundlach JH, Troll MA. (2006). Determination of RNA orientation during translocation through a biological nanopore. Biophys J, 90, 190–199.

Caron MP, Lafontaine DA, Massé E. (2010). Small RNA-mediated regulation at the level of transcript stability. RNA Biol, 7, 140–144.

Carpousis AJ. (2007). The RNA degradosome of Escherichia coli: an mRNA-degrading machine assembled on RNase E. Annu Rev Microbiol, 61, 71–87.

Chambers JR, Bender KS. (2011). The RNA chaperone Hfq is important for growth and stress tolerance in Francisella novicida. PLoS ONE, 6, e19797.

Chaulk SG, Smith Frieday MN, Arthur DC, Culham DE, Edwards RA, Soo P, Frost LS, Keates RA, Glover JN, Wood JM. (2011). ProQ is an RNA chaperone that controls ProP levels in Escherichia coli. Biochemistry, 50, 3095–3106.

Chiang MK, Lu MC, Liu LC, Lin CT, Lai YC. (2011). Impact of Hfq on global gene expression and virulence in Klebsiella pneumoniae. PLoS ONE, 6, e22248.

Christiansen JK, Larsen MH, Ingmer H, Søgaard-Andersen L, Kallipolitis BH. (2004). The RNA-binding protein Hfq of Listeria monocytogenes: role in stress tolerance and virulence. J Bacteriol, 186, 3355–3362.

Christiansen JK, Nielsen JS, Ebersbach T, Valentin-Hansen P, Søgaard-Andersen L, Kallipolitis BH. (2006). Identification of small Hfq-binding RNAs in Listeria monocytogenes. RNA, 12, 1383–1396.

Coenye T, Drevinek P, Mahenthiralingam E, Shah SA, Gill RT, Vandamme P, Ussery DW. (2007). Identification of putative noncoding RNA genes in the Burkholderia cenocepacia J2315 genome. FEMS Microbiol Lett, 276, 83–92.

Cohen-Or I, Shenhar Y, Biran D, Ron EZ. (2010). CspC regulates rpoS transcript levels and complements hfq deletions. Res Microbiol, 161, 694–700.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTS LINK
Copyright Clearance Center

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

Czech B, Hannon GJ. (2011). Small RNA sorting: matchmaking for Argonautes. Nat Rev Genet, 12, 19–31.
Davis BM, Waldor MK. (2007). RNase E-dependent processing stabilizes MicX, a Vibrio cholerae sRNA. Mol Microbiol, 65, 373–385.
de Haseth PL, Uhlenbeck OC. (1980a). Interaction of Escherichia coli host factor protein with oligoriboadenylates. Biochemistry, 19, 6138–6146.
de Haseth PL, Uhlenbeck OC. (1980b). Interaction of Escherichia coli host factor protein with Q beta ribonucleic acid. Biochemistry, 19, 6146–6151.
DiChiara JM, Contreras-Martinez LM, Livny J, Smith D, McDonough KA, Belfort M. (2010). Multiple small RNAs identified in Mycobacterium bovis BCG are also expressed in Mycobacterium tuberculosis and Mycobacterium smegmatis. Nucleic Acids Res, 38, 4067–4078.
Dienst D, Dühring U, Mollenkopf HJ, Vogel J, Golecki J, Hess WR, Wilde A. (2008). The cyanobacterial homologue of the RNA chaperone Hfq is essential for motility of Synechocystis sp. PCC 6803. Microbiology (Reading, Engl), 154, 3134–3143.
Diestra E, Cayrol B, Arluison V, Risco C. (2009). Cellular electron microscopy imaging reveals the localization of the Hfq protein close to the bacterial membrane. PLoS ONE, 4, e8301.
Dietrich M, Munke R, Gottschald M, Ziska E, Boettcher JP, Mollenkopf H, Friedrich A. (2009). The effect of hfq on global gene expression and virulence in Neisseria gonorrhoeae. FEBS J, 276, 5507–5520.
Ding Y, Davis BM, Waldor MK. (2004). Hfq is essential for Vibrio cholerae virulence and downregulates sigma expression. Mol Microbiol, 53, 345–354.
Drepper T, Raabe K, Giaourakis D, Gendrullis M, Masepohl B, Klipp W. (2002). The Hfq-like protein NrfA of the phototrophic purple bacterium Rhodobacter capsulatus controls nitrogen fixation via regulation of nifA and anfA expression. FEMS Microbiol Lett, 215, 221–227.
Dutta D, Bandyopadhyay K, Datta AB, Sardesai AA, Parrack P. (2009). Properties of HflX, an enigmatic protein from Escherichia coli. J Bacteriol, 191, 2307–2314.
Fantappiè L, Metruccio MM, Seib KL, Oriente F, Cartocci E, Ferlicca F, Giuliani MM, Scarlato V, Delany I. (2009). The RNA chaperone Hfq is involved in stress response and virulence in Neisseria meningitidis and is a pleiotropic regulator of protein expression. Infect Immun, 77, 1842–1853.
Felsenstein J. (1985). onfidence limits on phylogenies: An approach using the bootstrap. Evolution, 39, 783–791.
Fender A, Elf J, Hampel K, Zimmermann B, Wagner EG. (2010). RNAs actively cycle on the Sm-like protein Hfq. Genes Dev, 24, 2621–2626.
Folichon M, Allemand F, Régnier P, Hajnsdorf E. (2005). Stimulation of poly(A) synthesis by Escherichia coli poly(A)polymerase I is correlated with Hfq binding to poly(A) tails. FEBS J, 272, 454–463.
Franze de Fernandez MT, Eoyang L, August JT. (1968). Factor fraction required for the synthesis of bacteriophage Qbeta-RNA. Nature, 219, 588–590.
Franze de Fernandez MT, Hayward WS, August JT. (1972). Bacterial proteins required for replication of phage Q ribonucleic acid. Pruification and properties of host factor I, a ribonucleic acid-binding protein. J Biol Chem, 247, 824–831.
Fröhlich KS, Vogel J. (2009). Activation of gene expression by small RNA. Curr Opin Microbiol, 12, 674–682.
Geinguenaud F, Calandrini V, Teixeira J, Mayer C, Liquier J, Lavelle C, Arluison V. (2011). Conformational transition of DNA bound to Hfq probed by infrared spectroscopy. Phys Chem Chem Phys, 13, 1222–1229.
Geissmann T, Chevalier C, Cros MJ, Boisset S, Fechter P, Noirot C, Schrenzel J, François P, Vandenesch F, Gaspin C, Romby P. (2009). A search for small noncoding RNAs in Staphylococcus aureus reveals a conserved sequence motif for regulation. Nucleic Acids Res, 37, 7239–7257.
Geissmann TA, Touati D. (2004). Hfq, a new chaperoning role: binding to messenger RNA determines access for small RNA regulator. EMBO J, 23, 396–405.
Geng J, Song Y, Yang L, Feng Y, Qiu Y, Li G, Guo J, Bi Y, Qu Y, Wang W, Wang X, Guo Z, Yang R, Han Y. (2009). Involvement of the post-transcriptional regulator Hfq in Yersinia pestis virulence. PLoS ONE, 4, e6213.
Georg J, Hess WR. (2011). cis-antisense RNA, another level of gene regulation in bacteria. Microbiol Mol Biol Rev, 75, 286–300.
Guillier M, Gottesman S. (2008). The 5′ end of two redundant sRNAs is involved in the regulation of multiple targets, including their own regulator. Nucleic Acids Res, 36, 6781–6794.
Guisbert E, Rhodius VA, Ahuja N, Witkin E, Gross CA. (2007). Hfq modulates the sigmaE-mediated envelope stress response and the sigma32-mediated cytoplasmic stress response in Escherichia coli. J Bacteriol, 189, 1963–1973.
Hansen AM, Kaper JB. (2009). Hfq affects the expression of the LEE pathogenicity island in enterohaemorrhagic Escherichia coli. Mol Microbiol, 73, 446–465.
Hao Y, Zhang ZJ, Erickson DW, Huang M, Huang Y, Li J, Hwa T, Shi H. (2011). Quantifying the sequence-function relation in gene silencing by bacterial small RNAs. Proc Natl Acad Sci U S A, 108, 12473–12478.
Holmqvist E, Reimegård J, Sterk M, Grantcharova N, Römling U, Wagner EG. (2010). Two antisense RNAs target the transcriptional regulator CsgD to inhibit curli synthesis. EMBO J, 29, 1840–1850.
Hopkins JF, Panja S, Woodson SA. (2011). Rapid binding and release of Hfq from ternary complexes during RNA annealing. Nucleic Acids Res, 39, 5193–5202.
Huang HY, Chang HY, Chou CH, Tseng CP, Ho SY, Yang CD, Ju YW, Huang HD. (2009). sRNAMap: genomic maps for small non-coding RNAs, their regulators and their targets in microbial genomes. Nucleic Acids Res, 37, D150–D154.
Hussein R, Lim HN. (2011). Disruption of small RNA signaling caused by competition for Hfq. Proc Natl Acad Sci USA, 108, 1110–1115.
Hwang W, Arluison V, Hohng S. (2011). Dynamic competition of DsrA and rpoS fragments for the proximal binding site of Hfq as a means for efficient annealing. Nucleic Acids Res, 39, 5131–5139.
Ikeda Y, Yagi M, Morita T, Aiba H. (2011). 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, 79, 419–432.
Ishihama A. (1976). Proceedings: Control of RNA polymerase synthesis in Escherichia coli. J Biochem, 79, 33P–34P.
Ishihama Y, Schmidt T, Rappsilber J, Mann M, Hartl FU, Kerner MJ, Frishman D. (2008). Protein abundance profiling of the Escherichia coli cytosol. BMC Genomics, 9, 102.
Johansen J, Eriksen M, Kallipolitis B, Valentin-Hansen P. (2008). Down-regulation of outer membrane proteins by noncoding RNAs: unraveling the cAMP-CRP- and sigmaE-dependent CyaR-ompX regulatory case. J Mol Biol, 383, 1–9.
Kadowaki MA, Iulek J, Barbosa JA, Pedrosa Fde O, de Souza EM, Chubatsu LS, Monteiro RA, de Oliveira MA, Steffens MB. (2012). Structural characterization of the RNA chaperone Hfq from the nitrogen-fixing bacterium Herbaspirillum seropedicae SmR1. Biochim Biophys Acta, 1824, 359–365.
Kadzhaev K, Zingmark C, Golovliov I, Bolanowski M, Shen H, Conlan W, Sjöstedt A. (2009). Identification of genes contributing to the virulence of Francisella tularensis SCHU S4 in a mouse intradermal infection model. PLoS ONE, 4, e5463.
Kajitani M, Ishihama A. (1991). Identification and sequence determination of the host factor gene for bacteriophage Q beta. Nucleic Acids Res, 19, 1063–1066.
Kajitani M, Kato A, Wada A, Inokuchi Y, Ishihama A. (1994). Regulation of the Escherichia coli hfq gene encoding the host factor for phage Q beta. J Bacteriol, 176, 531–534.
Kaminski PA, Desnoues N, Elmerich C. (1994). The expression of nifA in Azorhizobium caulinodans requires a gene product homologous to Escherichia coli HF-I, an RNA-binding protein involved in the

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

replication of phage Q beta RNA. Proc Natl Acad Sci USA, 91, 4663–4667.

Kaminski PA, Elmerich C. (1998). The control of Azorhizobium caulinodans nifA expression by oxygen, ammonia and by the HF-I-like protein, NrfA. Mol Microbiol, 28, 603–613.

Kar S, Edgar R, Adhya S. (2005). Nucleoid remodeling by an altered HU protein: reorganization of the transcription program. Proc Natl Acad Sci USA, 102, 16397–16402.

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

Keates RA, Culham DE, Vernikovska YI, Zuiani AJ, Boggs JM, Wood JM. (2010). Transmembrane helix I and periplasmic loop 1 of Escherichia coli ProP are involved in osmosensing and osmoprotectant transport. Biochemistry, 49, 8847–8856.

Kulesus RR, Diaz-Perez K, Slechta ES, Eto DS, Mulvey MA. (2008). Impact of the RNA chaperone Hfq on the fitness and virulence potential of uropathogenic Escherichia coli. Infect Immun, 76, 3019–3026.

Lazar P, Lee Y, Kim S, Chandrasekaran M, Lee KW. (2010). Molecular Dynamics Simulation Study for Ionic Strength Dependence of RNA-host factor Interaction in Staphylococcus aureus Hfq. Bull Korean Chem Soc, 31, 1519–1526.

Le Derout J, Boni IV, Régnier P, Hajnsdorf E. (2010). Hfq affects mRNA levels independently of degradation. BMC Mol Biol, 11, 17.

Lease RA, Woodson SA. (2004). Cycling of the Sm-like protein Hfq on the DsrA small regulatory RNA. J Mol Biol, 344, 1211–1223.

Lee T, Feig AL. (2008). The RNA binding protein Hfq interacts specifically with tRNAs. RNA, 14, 514–523.

Link TM, Valentin-Hansen P, Brennan RG. (2009). Structure of Escherichia coli Hfq bound to polyriboadenylate RNA. Proc Natl Acad Sci USA, 106, 19292–19297.

Liu H, Wang Q, Liu Q, Cao X, Shi C, Zhang Y. (2011). Roles of Hfq in the stress adaptation and virulence in fish pathogen Vibrio alginolyticus and its potential application as a target for live attenuated vaccine. Appl Microbiol Biotechnol, 91, 353–364.

Liu JM, Livny J, Lawrence MS, Kimball MD, Waldor MK, Camilli A. (2009). Experimental discovery of sRNAs in Vibrio cholerae by direct cloning, 5S/tRNA depletion and parallel sequencing. Nucleic Acids Res, 37, e46.

Liu Y, Wu N, Dong J, Gao Y, Zhang X, Mu C, Shao N, Yang G. (2010). Hfq is a global regulator that controls the pathogenicity of *Staphylococcus aureus*. PLoS One, 5, pii: e13069.

Liveris D, Klotsky RA, Schwartz I. (1991). Growth rate regulation of translation initiation factor IF3 biosynthesis in Escherichia coli. J Bacteriol, 173, 3888–3893.

Livny J, Brencic A, Lory S, Waldor MK. (2006). Identification of 17 Pseudomonas aeruginosa sRNAs and prediction of sRNA-encoding genes in 10 diverse pathogens using the bioinformatic tool sRNAPredict2. Nucleic Acids Res, 34, 3484–3493.

Lorenz C, Gesell T, Zimmermann B, Schoeberl U, Bilusic I, Rajkowitsch L, Waldsich C, von Haeseler A, Schroeder R. (2010). Genomic SELEX for Hfq-binding RNAs identifies genomic aptamers predominantly in antisense transcripts. Nucleic Acids Res, 38, 3794–3808.

Lybecker MC, Abel CA, Feig AL, Samuels DS. (2010). Identification and function of the RNA chaperone Hfq in the Lyme disease spirochete Borrelia burgdorferi. Mol Microbiol, 78, 622–635.

Maki K, Morita T, Otaka H, Aiba H. (2010). A minimal base-pairing region of a bacterial small RNA SgrS required for translational repression of ptsG mRNA. Mol Microbiol, 76, 782–792.

Man S, Cheng R, Miao C, Gong Q, Gu Y, Lu X, Han F, Yu W. (2011). Artificial trans-encoded small non-coding RNAs specifically silence the selected gene expression in bacteria. Nucleic Acids Res, 39, e50.

Massé E, Escorcia FE, Gottesman S. (2003). Coupled degradation of a small regulatory RNA and its mRNA targets in Escherichia coli. Genes Dev, 17, 2374–2383.

McNealy TL, Forsbach-Birk V, Shi C, Marre R. (2005). The Hfq homolog in Legionella pneumophila demonstrates regulation by LetA and RpoS and interacts with the global regulator CsrA. J Bacteriol, 187, 1527–1532.

Mehta P, Goyal S, Wingreen NS. (2008). A quantitative comparison of sRNA-based and protein-based gene regulation. Mol Syst Biol, 4, 221.

Meibom KL, Forslund AL, Kuoppa K, Alkhuder K, Dubail I, Dupuis M, Forsberg A, Charbit A. (2009). Hfq, a novel pleiotropic regulator of virulence-associated genes in Francisella tularensis. Infect Immun, 77, 1866–1880.

Mellin JR, McClure R, Lopez D, Green O, Reinhard B, Genco C. (2010). Role of Hfq in iron-dependent and -independent gene regulation in Neisseria meningitidis. Microbiology (Reading, Engl), 156, 2316–2326.

Mikulecky PJ, Kaw MK, Brescia CC, Takach JC, Sledjeski DD, Feig AL. (2004). Escherichia coli Hfq has distinct interaction surfaces for DsrA, rpoS and poly(A) RNAs. Nat Struct Mol Biol, 11, 1206–1214.

Mitobe J, Morita-Ishihara T, Ishihama A, Watanabe H. (2009). Involvement of RNA-binding protein Hfq in the osmotic-response regulation of invE gene expression in Shigella sonnei. BMC Microbiol, 9, 110.

Mitschke J, Georg J, Scholz I, Sharma CM, Dienst D, Bantscheff J, Voss B, Steglich C, Wilde A, Vogel J, Hess WR. (2011). An experimentally anchored map of transcriptional start sites in the model cyanobacterium Synechocystis sp. PCC6803. Proc Natl Acad Sci USA, 108, 2124–2129.

Mohanty BK, Maples VF, Kushner SR. (2004). The Sm-like protein Hfq regulates polyadenylation dependent mRNA decay in Escherichia coli. Mol Microbiol, 54, 905–920.

Moll I, Leitsch D, Steinhauser T, Bläsi U. (2003). RNA chaperone activity of the Sm-like Hfq protein. EMBO Rep, 4, 284–289.

Møller T, Franch T, Højrup P, Keene DR, Bächinger HP, Brennan RG, Valentin-Hansen P. (2002). Hfq: a bacterial Sm-like protein that mediates RNA-RNA interaction. Mol Cell, 9, 23–30.

Moon K, Gottesman S. (2011). Competition among Hfq-binding small RNAs in Escherichia coli. Mol Microbiol, 82, 1545–1562.

Morita T, Maki K, Aiba H. (2005). RNase E-based ribonucleoprotein complexes: mechanical basis of mRNA destabilization mediated by bacterial noncoding RNAs. Genes Dev, 19, 2176–2186.

Morita T, Mochizuki Y, Aiba H. (2006). Translational repression is sufficient for gene silencing by bacterial small noncoding RNAs in the absence of mRNA destruction. Proc Natl Acad Sci USA, 103, 4858–4863.

Moskaleva O, Melnik B, Gabdulkhakov A, Garber M, Nikonov S, Stolboushkina E, Nikulin A. (2010). The structures of mutant forms of Hfq from Pseudomonas aeruginosa reveal the importance of the conserved His57 for the protein hexamer organization. Acta Crystallogr Sect F Struct Biol Cryst Commun, 66, 760–764.

Mulley G, White JP, Karunakaran R, Prell J, Bourdes A, Bunnewell S, Hill L, Poole PS. (2011). Mutation of GOGAT prevents pea bacteroid formation and N2 fixation by globally downregulating transport of organic nitrogen sources. Mol Microbiol, 80, 149–167.

Naidoo N, Harrop SJ, Sobti M, Haynes PA, Szymczyna BR, Williamson JR, Curmi PM, Mabbutt BC. (2008). Crystal structure of Lsm3 octamer from Saccharomyces cerevisiae: implications for Lsm ring organisation and recruitment. J Mol Biol, 377, 1357–1371.

Najima Y, Yahagi N, Takeuchi Y, Matsuzaka T, Sekiya M, Nakagawa Y, Amemiya-Kudo M, Okazaki H, Okazaki S, Tamura Y, Iizuka Y, Ohashi K, Harada K, Gotoda T, Nagai R, Kadowaki T, Ishibashi S, Yamada N, Osuga J, Shimano H. (2005). High mobility group protein-B1 interacts with sterol regulatory element-binding proteins to enhance their DNA binding. J Biol Chem, 280, 27523–27532.

Nakano M, Takahashi A, Su Z, Harada N, Mawatari K, Nakaya Y. (2008). Hfq regulates the expression of the thermostable direct hemolysin gene in Vibrio parahaemolyticus. BMC Microbiol, 8, 155.

Nei M, Kumar S. (2000). Molecular Evolution and Phylogenetics. New York: Oxford University Press.

Nielsen JS, Bøggild A, Andersen CB, Nielsen G, Boysen A, Brodersen DE, Valentin-Hansen P. (2007). An Hfq-like protein in archaea:

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK

crystal structure and functional characterization of the Sm protein from Methanococcus jannaschii. RNA, 13, 2213–2223.

Nielsen JS, Lei LK, Ebersbach T, Olsen AS, Klitgaard JK, Valentin-Hansen P, Kallipolitis BH. (2010). Defining a role for Hfq in Gram-positive bacteria: evidence for Hfq-dependent antisense regulation in Listeria monocytogenes. Nucleic Acids Res, 38, 907–919.

Nikulin A, Stolboushkina E, Perederina A, Vassilieva I, Blaesi U, Moll I, Kachalova G, Yokoyama S, Vassylyev D, Garber M, Nikonov S. (2005). Structure of Pseudomonas aeruginosa Hfq protein. Acta Crystallogr D Biol Crystallogr, 61, 141–146.

Ohniwa RL, Ushijima Y, Saito S, Morikawa K. (2011). Proteomic analyses of nucleoid-associated proteins in Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus. PLoS ONE, 6, e19172.

Olejniczak M. (2011). Despite similar binding to the Hfq protein regulatory RNAs widely differ in their competition performance. Biochemistry, 50, 4427–4440.

Olsen AS, Møller-Jensen J, Brennan RG, Valentin-Hansen P. (2010). C-terminally truncated derivatives of Escherichia coli Hfq are proficient in riboregulation. J Mol Biol, 404, 173–182.

Opdyke JA, Kang JG, Storz G. (2004). GadY, a small-RNA regulator of acid response genes in Escherichia coli. J Bacteriol, 186, 6698–6705.

Otaka H, Ishikawa H, Morita T, Aiba H. (2011). PolyU tail of rho-independent terminator of bacterial small RNAs is essential for Hfq action. Proc Natl Acad Sci USA, 108, 13059–13064.

Pandey SP, Minesinger BK, Kumar J, Walker GC. (2011). A highly conserved protein of unknown function in Sinorhizobium meliloti affects sRNA regulation similar to Hfq. Nucleic Acids Res, 39, 4691–4708.

Papenfort K, Bouvier M, Mika F, Sharma CM, Vogel J. (2010). Evidence for an autonomous 5′ target recognition domain in an Hfq-associated small RNA. Proc Natl Acad Sci USA, 107, 20435–20440.

Papenfort K, Pfeiffer V, Mika F, Lucchini S, Hinton JC, Vogel J. (2006). SigmaE-dependent small RNAs of Salmonella respond to membrane stress by accelerating global omp mRNA decay. Mol Microbiol, 62, 1674–1688.

Papenfort K, Vogel J. (2010). Regulatory RNA in bacterial pathogens. Cell Host Microbe, 8, 116–127.

Pfeiffer V, Papenfort K, Lucchini S, Hinton JC, Vogel J. (2009). Coding sequence targeting by MicC RNA reveals bacterial mRNA silencing downstream of translational initiation. Nat Struct Mol Biol, 16, 840–846.

Pontiggia A, Negri A, Beltrame M, Bianchi ME. (1993). Protein HU binds specifically to kinked DNA. Mol Microbiol, 7, 343–350.

Postic G, Frapy E, Dupuis M, Dubail I, Livny J, Charbit A, Meibom KL. (2010). Identification of small RNAs in Francisella tularensis. BMC Genomics, 11, 625.

Prévost K, Salvail H, Desnoyers G, Jacques JF, Phaneuf E, Massé E. (2007). The small RNA RyhB activates the translation of shiA mRNA encoding a permease of shikimate, a compound involved in siderophore synthesis. Mol Microbiol, 64, 1260–1273.

Rabhi M, Espéli O, Schwartz A, Cayrol B, Rahmouni AR, Arluison V, Boudvillain M. (2011). The Sm-like RNA chaperone Hfq mediates transcription antitermination at Rho-dependent terminators. EMBO J, 30, 2805–2816.

Raghavan R, Groisman EA, Ochman H. (2011). Genome-wide detection of novel regulatory RNAs in E. coli. Genome Res, 21, 1487–1497.

Ramos CG, Sousa SA, Grilo AM, Feliciano JR, Leitão JH. (2011). The second RNA chaperone, Hfq2, is also required for survival under stress and full virulence of Burkholderia cenocepacia J2315. J Bacteriol, 193, 1515–1526.

Robertson GT, Roop RM Jr. (1999). The Brucella abortus host factor I (HF-I) protein contributes to stress resistance during stationary phase and is a major determinant of virulence in mice. Mol Microbiol, 34, 690–700.

Saitou N, Nei M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol, 4, 406–425.

Salim NN, Feig AL. (2010). An upstream Hfq binding site in the fhlA mRNA leader region facilitates the OxyS-fhlA interaction. PLoS ONE, 5, pii: e13028.

Santos JM, Freire P, Mesquita FS, Mika F, Hengge R, Arraiano CM. (2006). Poly(A)-polymerase I links transcription with mRNA degradation via sigmaS proteolysis. Mol Microbiol, 60, 177–188.

Sauer E, Weichenrieder O. (2011). Structural basis for RNA 3′-end recognition by Hfq. Proc Natl Acad Sci USA, 108, 13065–13070.

Sauter C, Basquin J, Suck D. (2003). Sm-like proteins in Eubacteria: the crystal structure of the Hfq protein from Escherichia coli. Nucleic Acids Res, 31, 4091–4098.

Schiano CA, Bellows LE, Lathem WW. (2010). The small RNA chaperone Hfq is required for the virulence of Yersinia pseudotuberculosis. Infect Immun, 78, 2034–2044.

Schilling D, Gerischer U. (2009). The Acinetobacter baylyi Hfq gene encodes a large protein with an unusual C terminus. J Bacteriol, 191, 5553–5562.

Schlüter JP, Reinkensmeier J, Daschkey S, Evguenieva-Hackenberg E, Janssen S, Jänicke S, Becker JD, Giegerich R, Becker A. (2010). A genome-wide survey of sRNAs in the symbiotic nitrogen-fixing α-proteobacterium Sinorhizobium meliloti. BMC Genomics, 11, 245.

Schumacher MA, Pearson RF, Møller T, Valentin-Hansen P, Brennan RG. (2002). Structures of the pleiotropic translational regulator Hfq and an Hfq-RNA complex: a bacterial Sm-like protein. EMBO J, 21, 3546–3556.

Schuppli D, Miranda G, Tsui HC, Winkler ME, Sogo JM, Weber H. (1997). Altered 3′-terminal RNA structure in phage Qbeta adapted to host factor-less Escherichia coli. Proc Natl Acad Sci USA, 94, 10239–10242.

Senear AW, Steitz JA. (1976). Site-specific interaction of Qbeta host factor and ribosomal protein S1 with Qbeta and R17 bacteriophage RNAs. J Biol Chem, 251, 1902–1912.

Sharma CM, Darfeuille F, Plantinga TH, Vogel J. (2007). A small RNA regulates multiple ABC transporter mRNAs by targeting C/A-rich elements inside and upstream of ribosome-binding sites. Genes Dev, 21, 2804–2817.

Sharma CM, Hoffmann S, Darfeuille F, Reignier J, Findeiss S, Sittka A, Chabas S, Reiche K, Hackermüller J, Reinhardt R, Stadler PF, Vogel J. (2010). The primary transcriptome of the major human pathogen Helicobacter pylori. Nature, 464, 250–255.

Shields MJ, Fischer JJ, Wieden HJ. (2009). Toward understanding the function of the universally conserved GTPase HflX from Escherichia coli: a kinetic approach. Biochemistry, 48, 10793–10802.

Shimoni Y, Friedlander G, Hetzroni G, Niv G, Altuvia S, Biham O, Margalit H. (2007). Regulation of gene expression by small non-coding RNAs: a quantitative view. Mol Syst Biol, 3, 138.

Sittka A, Pfeiffer V, Tedin K, Vogel J. (2007). The RNA chaperone Hfq is essential for the virulence of Salmonella typhimurium. Mol Microbiol, 63, 193–217.

Sittka A, Sharma CM, Rolle K, Vogel J. (2009). 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, 6, 266–275.

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

Sobrero P, Valverde C. (2011). Evidences of autoregulation of hfq expression in Sinorhizobium meliloti strain 2011. Arch Microbiol, 193, 629–639.

Someya T, Baba S, Fujimoto M, Kawai G, Kumasaka T, Nakamura K. (2011). 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, 40, 1856–1867.

Sonnleitner E, Hagens S, Rosenau F, Wilhelm S, Habel A, Jäger KE, Bläsi U. (2003). Reduced virulence of a hfq mutant of Pseudomonas aeruginosa O1. Microb Pathog, 35, 217–228.

Sonnleitner E, Moll I, Bläsi U. (2002). Functional replacement of the Escherichia coli hfq gene by the homologue of Pseudomonas aeruginosa. Microbiology (Reading, Engl), 148, 883–891.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

© 2012 Informa Healthcare USA, Inc.

RIGHTSLINK

Soper T, Mandin P, Majdalani N, Gottesman S, Woodson SA. (2010). Positive regulation by small RNAs and the role of Hfq. Proc Natl Acad Sci USA, 107, 9602–9607.

Soper TJ, Doxzen K, Woodson SA. (2011). Major role for mRNA binding and restructuring in sRNA recruitment by Hfq. RNA, 17, 1544–1550.

Soper TJ, Woodson SA. (2008). The rpoS mRNA leader recruits Hfq to facilitate annealing with DsrA sRNA. RNA, 14, 1907–1917.

Sorger-Domenigg T, Sonnleitner E, Kaberdin VR, Bläsi U. (2007). Distinct and overlapping binding sites of Pseudomonas aeruginosa Hfq and RsmA proteins on the non-coding RNA RsmY. Biochem Biophys Res Commun, 352, 769–773.

Sousa SA, Ramos CG, Moreira LM, Leitão JH. (2010). The hfq gene is required for stress resistance and full virulence of Burkholderia cepacia to the nematode Caenorhabditis elegans. Microbiology (Reading, Engl), 156, 896–908.

Sukhodolets MV, Garges S. (2003). Interaction of Escherichia coli RNA polymerase with the ribosomal protein S1 and the Sm-like ATPase Hfq. Biochemistry, 42, 8022–8034.

Sun X, Wartell RM. (2006). Escherichia coli Hfq binds A18 and DsrA domain II with similar 2:1 Hfq6/RNA stoichiometry using different surface sites. Biochemistry, 45, 4875–4887.

Sun X, Zhulin I, Wartell RM. (2002). Predicted structure and phyletic distribution of the RNA-binding protein Hfq. Nucleic Acids Res, 30, 3662–3671.

Swiercz JP, Hindra, Bobek J, Bobek J, Haiser HJ, Di Berardo C, Tjaden B, Elliot MA. (2008). Small non-coding RNAs in Streptomyces coelicolor. Nucleic Acids Res, 36, 7240–7251.

Takada A, Wachi M, Kaidow A, Takamura M, Nagai K. (1997). DNA binding properties of the hfq gene product of Escherichia coli. Biochem Biophys Res Commun, 236, 576–579.

Talukder AA, Hossain MA, Yamada M, Ishihama A. (2006). Nucleoids Dynamic in Escherichia coli: A Growth Phase Dependent Process. Bangladesh J Microbiol, 23, 81–88.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011). MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol, 28, 2731–2739.

Tantos A, Han KH, Tompa P. (2012). Intrinsic disorder in cell signaling and gene transcription. Mol Cell Endocrinol, 348, 457–465.

Tolstorukov MY, Virnik KM, Adhya S, Zhurkin VB. (2005). A-tract clusters may facilitate DNA packaging in bacterial nucleoid. Nucleic Acids Res, 33, 3907–3918.

Torres-Quesada O, Oruezabal RI, Peregrina A, Jofré E, Lloret J, Rivilla R, Toro N, Jiménez-Zurdo JI. (2010). The Sinorhizobium meliloti RNA chaperone Hfq influences central carbon metabolism and the symbiotic interaction with alfalfa. BMC Microbiol, 10, 71.

Tsui HC, Feng G, Winkler ME. (1996). Transcription of the mutL repair, miaA tRNA modification, hfq pleiotropic regulator, and hflA region protease genes of Escherichia coli K-12 from clustered Esigma32-specific promoters during heat shock. J Bacteriol, 178, 5719–5731.

Tsui HC, Leung HC, Winkler ME. (1994). Characterization of broadly pleiotropic phenotypes caused by an hfq insertion mutation in Escherichia coli K-12. Mol Microbiol, 13, 35–49.

Tsui HC, Mukherjee D, Ray VA, Sham LT, Feig AL, Winkler ME. (2010). Identification and characterization of noncoding small RNAs in Streptococcus pneumoniae serotype 2 strain D39. J Bacteriol, 192, 264–279.

Tsui HC, Winkler ME. (1994). Transcriptional patterns of the mutL-miaA superoperon of Escherichia coli K-12 suggest a model for posttranscriptional regulation. Biochimie, 76, 1168–1177.

Updegrove TB, Correia JJ, Chen Y, Terry C, Wartell RM. (2011). The stoichiometry of the Escherichia coli Hfq protein bound to RNA. RNA, 17, 489–500.

Updegrove TB, Correia JJ, Galletto R, Bujalowski W, Wartell RM. (2010). E. coli DNA associated with isolated Hfq interacts with Hfq's distal surface and C-terminal domain. Biochim Biophys Acta, 1799, 588–596.

Updegrove TB, Wartell RM. (2011). The influence of Escherichia coli Hfq mutations on RNA binding and sRNA•mRNA duplex formation in rpoS riboregulation. Biochim Biophys Acta, 1809, 532–540.

Valentin-Hansen P, Eriksen M, Udesen C. (2004). The bacterial Sm-like protein Hfq: a key player in RNA transactions. Mol Microbiol, 51, 1525–1533.

Valverde C, Livny J, Schlüter JP, Reinkensmeier J, Becker A, Parisi G. (2008). Prediction of Sinorhizobium meliloti sRNA genes and experimental detection in strain 2011. BMC Genomics, 9, 416.

Vanderpool CK, Gottesman S. (2004). Involvement of a novel transcriptional activator and small RNA in post-transcriptional regulation of the glucose phosphoenolpyruvate phosphotransferase system. Mol Microbiol, 54, 1076–1089.

Vecerek B, Beich-Frandsen M, Resch A, Bläsi U. (2010). Translational activation of rpoS mRNA by the non-coding RNA DsrA and Hfq does not require ribosome binding. Nucleic Acids Res, 38, 1284–1293.

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

Vecerek B, Rajkowitsch L, Sonnleitner E, Schroeder R, Bläsi U. (2008). The C-terminal domain of Escherichia coli Hfq is required for regulation. Nucleic Acids Res, 36, 133–143.

Verdone L, Galardi S, Page D, Beggs JD. (2004). Lsm proteins promote regeneration of pre-mRNA splicing activity. Curr Biol, 14, 1487–1491.

Verrier CS, Roodi N, Yee CJ, Bailey LR, Jensen RA, Bustin M, Parl FF. (1997). High-mobility group (HMG) protein HMG-1 and TATA-binding protein-associated factor TAF(II)30 affect estrogen receptor-mediated transcriptional activation. Mol Endocrinol, 11, 1009–1019.

Viegas SC, Arraiano CM. (2008). Regulating the regulators: How ribonucleases dictate the rules in the control of small non-coding RNAs. RNA Biol, 5, 230–243.

Viegas SC, Pfeiffer V, Sittka A, Silva IJ, Vogel J, Arraiano CM. (2007). Characterization of the role of ribonucleases in Salmonella small RNA decay. Nucleic Acids Res, 35, 7651–7664.

Vogel J, Luisi BF. (2011). Hfq and its constellation of RNA. Nat Rev Microbiol, 9, 578–589.

Vytvytska O, Jakobsen JS, Balcunaite G, Andersen JS, Baccarini M, von Gabain A. (1998). Host factor I, Hfq, binds to Escherichia coli ompA mRNA in a growth rate-dependent fashion and regulates its stability. Proc Natl Acad Sci USA, 95, 14118–14123.

Wang W, Wang L, Zou Y, Zhang J, Gong Q, Wu J, Shi Y. (2011). Cooperation of Escherichia coli Hfq hexamers in DsrA binding. Genes Dev, 25, 2106–2117.

Wilson KS, von Hippel PH. (1995). Transcription termination at intrinsic terminators: the role of the RNA hairpin. Proc Natl Acad Sci USA, 92, 8793–8797.

Windbichler N, von Pelchrzim F, Mayer O, Csaszar E, Schroeder R. (2008). Isolation of small RNA-binding proteins from E. coli: evidence for frequent interaction of RNAs with RNA polymerase. RNA Biol, 5, 30–40.

Wu XG, Duan HM, Tian T, Yao N, Zhou HY, Zhang LQ. (2010). Effect of the hfq gene on 2,4-diacetylphloroglucinol production and the PcoI/PcoR quorum-sensing system in Pseudomonas fluorescens 2P24. FEMS Microbiol Lett, 309, 16–24.

Zaric B, Chami M, Rémigy H, Engel A, Ballmer-Hofer K, Winkler FK, Kambach C. (2005). Reconstitution of two recombinant LSm protein complexes reveals aspects of their architecture, assembly, and function. J Biol Chem, 280, 16066–16075.

Zhang A, Wassarman KM, Ortega J, Steven AC, Storz G. (2002). The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. Mol Cell, 9, 11–22.

Zhang A, Wassarman KM, Rosenow C, Tjaden BC, Storz G, Gottesman S. (2003). Global analysis of small RNA and mRNA targets of Hfq. Mol Microbiol, 50, 1111–1124.

Zhang Y, Hong G. (2009). Post-transcriptional regulation of NifA expression by Hfq and RNase E complex in Rhizobium leguminosarum bv. viciae. Acta Biochim Biophys Sin (Shanghai), 41, 719–730.

Critical Reviews in Microbiology Downloaded from informahealthcare.com by 200.63.1.193 on 03/21/12
For personal use only.

RIGHTSLINK