Biochimie xxx (2012) 1–6

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Lack of interchangeability of Hfq-like proteins

Tatiana Rochat [a], Philippe Bouloc [a], Qi Yang [b], Lionello Bossi [b], Nara Figueroa-Bossi [b,*]

[a] Institut de Génétique et Microbiologie, CNRS, UMR 8621, IFR115, Université Paris-Sud, Orsay, France
[b] Centre de Génétique Moléculaire, CNRS, UPR3404, IFR115, Gif-sur-Yvette, France

ARTICLE INFO

Article history:
Received 8 December 2011
Accepted 20 January 2012
Available online xxx

Keywords:
Hfq
Salmonella
Small RNA
Staphylococcus aureus
Borrelia burgdorferi

ABSTRACT

Hfq is an RNA-binding protein that participates in the regulatory activity of small non-coding RNAs (sRNAs) in many species of bacteria. Hfq protein was first crystallized from *Staphylococcus aureus* and this crystal structure constitutes a hallmark for bacterial Sm-like proteins. Paradoxically, however, the functional relevance/role of *S. aureus* Hfq (Hfq_{SA}) remains uncertain, as growing evidence suggests that the hfq_{SA} gene is expressed at very low levels or unexpressed in many *S. aureus* strains. To gather further insight, in the present work we exchanged the structural portion of the *hfq* gene of *Salmonella enterica* serovar Typhimurium (hfq_{STM}) with hfq_{SA} and analyzed the effects of the replacement on various Hfq-related phenotypes. Our results show that the replacement strain – in spite of expressing Hfq_{SA} at levels comparable to Hfq_{STM} in wild-type *Salmonella* – behaves as an *hfq* null mutant in three discrete small RNA-mediated regulatory responses. These defects correlate with an abrupt reduction in the intracellular concentration of sRNAs, as observed in an *hfq* null mutant. Failure of Hfq_{SA} to protect *Salmonella* sRNAs from degradation suggests that Hfq_{Sa} does not bind to these sRNAs. A parallel study with the *Borrelia burgdorferi hfq* gene (hfq_{BB}) gave essentially identical results: when made from a single copy chromosomal gene, Hfq_{BB} fails to substitute for Hfq_{STM} in sRNA-mediated regulation.

1. Introduction

Base pairing between regulatory RNAs and complementary sequences in messenger RNAs is a highly conserved mechanism controlling gene expression at the post-transcriptional level in all forms of life. In bacteria, best-studied regulatory small RNAs (sRNAs) are encoded at separate locations from their target genes and interact with target mRNAs through short and imperfect stretches of complementarity (reviewed in [1]). In some bacterial species, like *Escherichia coli*, *Salmonella enterica* and *Vibrio* species, these *trans*-encoded sRNAs require chaperon protein Hfq for activity. Discovered in the late nineteen sixties as a host factor needed for in vitro replication of RNA phage Qß [2], Hfq was later found to be a key player in a number of RNA transactions (reviewed in [3–5]). In particular, Hfq participates in sRNA-mediated regulation by binding both sRNAs and cognate mRNAs and stimulating their association. For many sRNAs, Hfq-binding is also essential to confer protection against degradation by ribonucleases [6]. Hfq and Hfq-like proteins belong to the Sm-like (Lsm) family of RNA-bind ing proteins characterized by a ring-shaped multimeric architecture. X-ray crystal structure analysis of the Hfq-like protein from *Staphylococcus aureus* (Hfq_{SA}) and of *E. coli* Hfq (Hfq_{EC}) showed that both proteins assemble in homohexameric rings approximately 70 Å in diameter [7–9]. A short U-rich synthetic RNA co-crystallized with Hfq_{SA} was found to circle around the positively charged central pore of the torus on the so-called proximal face [9]. In contrast, A-containing RNA oligomers bind to the distal face of Hfq_{EC} [7]. The notion of opposite Hfq surfaces having different ligand specificities is independently supported by mutational studies [10]. It should be noticed, however, that while similar on the proximal face, Hfq from *S. aureus* and *E. coli* differ sharply in their charge distribution on the distal face and in the trough that connects proximal and distal faces. This latter region has a positively charged surface in Hfq_{EC} and a negatively charged surface in Hfq_{SA} [3]. Finally, while Hfq-like proteins have an evolutionarily conserved core of 65 amino acids, the C-terminus is variable in length, leading to a controversy about its function [11,12]. The Hfq extended C-terminus is found in γ- and β-proteobacteria whereas in the case of Gram-positive bacteria such as in Hfq_{SA}, extensions are short.

In most bacteria, loss of Hfq function, albeit not a lethal event, causes a variety of pleiotropic defects and renders strains particularly susceptible to environmental stress [13]. Many of these phenotypes result from the loss of sRNA activities. For example, the σ^S-dependent stress response is poorly induced in *hfq* mutants due

* Corresponding author. Centre de Génétique Moléculaire-CNRS, 1 Av de la Terrasse, 91198 Gif-sur-Yvette cedex, France. Tel.: + 33 169 823 811; fax: +33 169 823 877.
E-mail address: figueroa@cgm.cnrs-gif.fr (N. Figueroa-Bossi).

0300-9084/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2012.01.016

to the lack of sRNA-mediated activation of *rpoS* mRNA translation (reviewed in [14]). At the same time, the σ^E-dependent envelope stress response is chronically induced due to over-accumulation of outer membrane proteins that are normally downregulated by sRNAs [15–20].

Genes encoding Hfq-like proteins are found in about half of the sequenced genomes [21]. In many pathogens, they are required for virulence and were shown to participate in sRNA-mediated regulatory processes (reviewed in [22]). The functional proficiency of Hfq homologues was inferred from their ability to complement the loss of Hfq in *E. coli*. For example, *Pseudomonas aeruginosa* Hfq, which shares 92% identity with Hfq_{EC} in the initial 68 amino acids, fully replaced Hfq_{Ec} in terms of its requirement for Qβ replication and *rpoS* expression [23]. Likewise, the *Moraxella catarrhali hfq*-like gene, in spite of being twice the size of the hfq_{EC} (but highly similar in the N-terminal encoding domain) partially complemented the growth defect and the stress sensitivity of an *E. coli hfq* mutant [24]. Intriguingly, even a protein with very limited sequence relatedness to Hfq (only 12% identity), encoded by *Borrelia burgdorferi* (Hfq_{BB}), was recently reported to complement an *E. coli hfq* mutant [25]. On the other hand, Hfq-like proteins from *Neisseria meningitides* and *Aquifex aeolicus,* and from archaeon *Methanocaldococcus jannaschii* did not reverse the chronic σ^E induction of a *Salmonella* strain lacking Hfq [26]. However, these proteins were capable of binding some *Salmonella* sRNAs and also caused specific RNA processing defects [26].

The function of Hfq-like proteins remains unclear for some bacterial species. For example, deletion of the *Bacillus subtilis hfq*-like gene (*ymaH*) does not affect growth rate, stress adaptation, or activities of all sRNAs tested [27–29]. Similar results were reported for *S. aureus*. Deletion of the hfq_{SA} gene in several pathogenic isolates did not impair or in any way impact their physiology [30] possibly because hfq_{SA} is poorly expressed or not expressed in the strains used for these studies [30,31](see also [32]). On the other hand, in *S. aureus* strains where Hfq is detected, deletion of its coding gene reportedly resulted in decreased toxicity and virulence, suggesting that Hfq is a global regulator [33].

To gather insight into the functional status and regulatory properties of *S. aureus* Hfq, in the present study, we introduced the sequence encoding this protein in place of the endogenous *hfq* gene in the *Salmonella* chromosome. (Hfq_{STM} is 100% identical to Hfq_{EC} in the initial 78 amino acids). In parallel with the above work, a similar exchange was performed using a DNA fragment spanning the *Borrelia hfq*-like gene. Both constructs were made with surgical precision replacing only protein coding portions – *i.e*., the segment between initiation and termination codons – to allow the heterologous sequences to fall under the control of signals normally devoted to hfq_{STM} expression. We show below that Hfq_{SA} and Hfq_{BB} in spite of being made at levels comparable to Hfq_{STM}, fail to replace the latter in sRNA-mediated regulation as well as in protecting representative sRNAs from degradation.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Strains used in this study, listed in Table 1, were all derived from *S. enterica* serovar Typhimurium strain MA3409, a derivative of strain LT2 cured for the Gifsy-1 prophage [34]. Bacteria were cultured at 37 °C in liquid media or in media solidified by the addition of 1.5% (w/v) Difco agar. LB broth [1% bacto tryptone (w/v), 0.5% Difco yeast extract (w/v), 0.5% NaCl (w/v)] was used as complex medium. When needed, LB medium was supplemented with 0.2% (w/v) arabinose. Antibiotics (Sigma–Aldrich) were included at the following final concentrations: chloramphenicol, 10 μg/ml; kanamycin monosulphate, 50 μg/ml; sodium ampicillin 100 μg/ml; tetracycline hydrochloride, 25 μg/ml. Liquid cultures were grown in New Brunswick gyratory shakers and growth was monitored by measuring the optical density at 600 nm with a Shimazu UV-mini 1240 spectrophotometer.

Table 1
Salmonella enterica serovar Typhimurium strains used in this work.

Strain[a]	Genotype	Source or reference
MA3409	wild-type	[34]
MA7455	wild-type/pKD46	[18]
MA8028	*eptB115*::MudK	[18]
MA8029	*eptB115*::MudK Δ*hfq67*::*cat*	[18]
MA8149	*katE561*::MudK	[18]
MA8679	*katE561*::MudK Δ*hfq67*::*cat*	[18]
MA9132	*chiP91*::pCE40(*lac*)	[43]
MA10675	Δ*hfq116*::*tetAR*	this work
MA10740	Δ*hfq116*::*tetAR*/pKD46	this work
MA10741	Δhfq_{STM}::hfq_{SA}	this work
MA10744	*chiP91*::pCE40(*lac*) Δ*hfq116*::*tetAR*	this work
MA10746	*eptB115*::MudK Δhfq_{STM}::hfq_{SA}	this work
MA10747	*chiP91*::pCE40(*lac*) Δhfq_{STM}::hfq_{SA}	this work
MA11042	Δhfq_{STM}::hfq_{BB}	this work
MA11043	*eptB115*::MudK Δhfq_{STM}::hfq_{BB}	this work
MA11044	*chiP91*::pCE40(*lac*) Δhfq_{STM}::hfq_{BB}	this work
MA11054	*hfq*-3xFLAG-*aph* (KnR)	this work
MA11055	Δhfq_{STM}::hfq_{SA}-3xFLAG-*aph* (KnR)	this work
MA11056	Δhfq_{STM}::hfq_{BB}-3xFLAG-*aph* (KnR)	this work
MA11057	*katE561*::MudK Δhfq_{STM}::hfq_{SA}	this work
MA11058	*katE561*::MudK Δhfq_{STM}::hfq_{BB}	this work

[a] All strains are derived from *Salmonella enterica* serovar Typhimurium strain MA3409. The latter is a derivative of strain LT2 cured for the Gifsy-1 prophage [34].

2.2. Enzymes and chemicals

Restriction enzymes, T4 polynucleotide kinase and Taq DNA polymerase were from New England Biolabs, Pfu-Turbo DNA polymerase was from Stratagene. DNA oligonucleotides were obtained from Sigma–Aldrich. Acrylamide-bisacrylamide (30%, 29:1) and other electrophoresis reagents were from Bio-Rad. Hybond-N^+ membranes and hybridization buffer used for Northern blot analysis were from GE Healthcare and from Applied Biosystems-Ambion, respectively.

2.3. Genetic techniques

Generalized transduction was carried out using the high frequency transducing mutant of phage P22, HT 105/1 *int-201* [35]. "λ Red"-mediated chromosomal recombineering was carried out by the method of Datsenko and Wanner [36] implemented as in [37]. Donor DNA fragments were generated by the polymerase chain reaction (PCR) using plasmid or chromosomal DNA templates. DNA oligonucleotides used as primers for PCR amplification are listed in Table 2. Amplified fragments were electroporated into the appropriate strains using a Bio-Rad MicroPulser under the conditions specified by the manufacturer. Constructs were verified by PCR and DNA sequence analysis (performed by GATC company).

2.4. Construction of relevant strains

Salmonella strains carrying the structural portions of the *hfq*-like genes from *S. aureus* or *B. burgdorferi* were constructed with a two-step recombineering procedure as described [38]. Firstly, a *tetAR* module (amplified with primers ppH71 and ppH72) was inserted in the *hfq* gene in the *Salmonella* chromosome. Subsequently, the entire *hfq*::*tetAR* was crossed out selecting for the loss of tetracycline resistance [39] using DNA fragments amplified from chromosomal DNA of *S. aureus* RN4220 and *B. burgdorferi* clinical isolate 28354 with primer pairs ppI06/ppI07 and ppJ44/ppJ45, respectively

Please cite this article in press as: T. Rochat, et al., Lack of interchangeability of Hfq-like proteins, Biochimie (2012), doi:10.1016/j.biochi.2012.01.016

Table 2
DNA oligonucleotides used as PCR primers for λ Red-mediated recombination.

Primer[a]	*Sequence* (5'– 3')
ppH71(fw)	ATTGCGTCGGGAACGTGTTCCAGTTTCTATTTATTTGGTG*TTAAGACCCACTTTCACATT*
ppH72 (rv)	AACTGATCAAAGGACTCGATTTGACCTTGCAGCTTAATACC*CTAAGCACTTGTCTCCTG*
ppI06 (fw)	AGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGA*ATGATTGCAAACGAAAACATCCA*
ppI07 (rv)	ATTATCCGACGCCCCCGACATGGATAAACAGCGCGTGAAC*TTATTCTTCACTTTCAGTAGATGC*
ppJ44 (fw)	AAAGGTTCAAAGTACAAATAAGCATATAAGGAAAAGAGA*ATGTTTATAAGCAGGGAATTGAAG*
ppJ45 (rv)	ATTATCCGACGCCCCCGACATGGATAAACAGCGCGTGAAC*TTATTCCTTCTTGCTCATTAAAG*
pp913 (fw)	GCAGGGGTCTACTGCGCAACAGGACAGCGAAGAGACTGAA*GACTACAAAGACCATGACGG*
pp914 (rv)	ATTATCCGACGCCCCCGACATGGATAAACAGCGCGTGAAC*CATATGAATATCCTCCTTAG*
ppJ40 (fw)	AAAGTTGTTGCAGATGCTATTAAAACTTTAATGAGCAAGAAGGAA*GACTACAAAGACCATGACGG*
pp914 (rv)	above
ppJ41 (fw)	TATACAGTAGAAACTGAAGGTCAAGCATCTACTGAAAGTGAAGAA*GACTACAAAGACCATGACGG*
pp914 (rv)	above

[a] Primers are defined as "forward" (fw) or "reverse" (rv) depending on whether they have same or opposite orientation (5'-3') relative to the gene being modified. The portions of primers annealing to template DNA are in red italics.

(Table 2). Introduction of the 3xFLAG epitope at the 3′ ends of the coding sequence of hfq_{STM}, hfq_{SA} and hfq_{BB} was carried out using DNA fragments amplified from plasmid pSUB11 [37] with primer pairs pp913/pp914, ppJ41/pp914 and ppJ40/pp914, respectively, as previously described [37].

2.5. RNA extraction and Northern blot analysis

RNA was prepared by the acid-hot-phenol method from exponentially growing cells (OD_{600} of 0.35) as previously described [40]. RNA was separated on an 8% polyacrylamide-8 M urea gel and electro-blotted onto a nylon membrane. Blots were hybridized to 5′ end-labelled DNA oligonucleotide probes specific for the sRNAs under study (listed in Table 3). Hybridization signals were analyzed by Phosphorimaging using ImageQuant software.

Table 3
DNA oligonucleotides used as probes for Northern analysis.

Name	Sequence (5′–3′)	Specificity
pp814	ATGATGATAACAAATGCGCG	MicA
pp831	AGGTTAACGCAATGGCCCAG	MicC
pp832	AGGGGTAAACAGACATTCAG	MicF
ppB07	CGTCAAAGAGGAATTTCATCGTTATTATTATC CCGACGCTTTCGCTTC	ChiX
ppB10	ACACTACCATCGGCGCTACG	5 S
ppI67	AGACCAATTGCAAACACAACAACACAACATC ACAACCGTAAGCCA	GcvB

2.6. Western blot analysis

Bacterial cells harvested by centrifugation were resuspended in 250 μl of Laemmli protein gel loading buffer. Whole-cell extracts were fractioned in a 15% polyacrylamide-SDS gel. Proteins were transferred to poly-vinylidene difluoride (PVDF) membranes and probed with anti-FLAG M2 monoclonal antibodies from Sigma. Horse Radish Peroxidase (HRP) conjugated goat anti-mouse IgG (KPL) was used as a secondary antibody. Detection was performed by Enhanced Chemioluminescence (ECL, Amersham) with ECL films (Amersham).

2.7. β-galactosidase assays

Activity of β-galactosidase was measured in toluene-permeabilized cells as described [41] and is expressed in Miller units. Reported values were the average of at least two independent determinations, each involving duplicate or triplicate samples.

3. Results and discussion

3.1. Exchanging hfq coding sequences

Replacement of the structural portion of the *Salmonella hfq* gene with the corresponding regions from the *hfq* genes of *S. aureus* and *B. burgdorferi* was achieved by a two-step recombineering procedure (see Materials and Methods). DNA sequence analysis of the

Please cite this article in press as: T. Rochat, et al., Lack of interchangeability of Hfq-like proteins, Biochimie (2012), doi:10.1016/j.biochi.2012.01.016

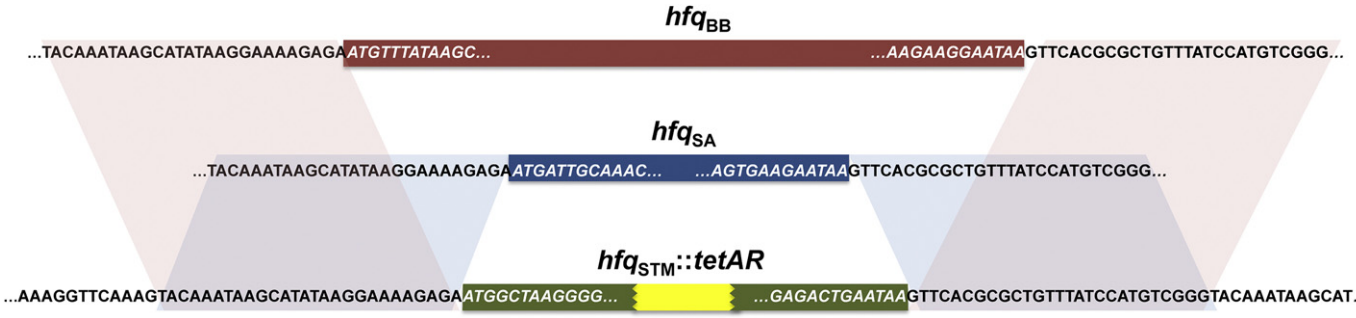

Fig. 1. Schematic representation of recombineering events replacing the coding segment of the *hfq* gene in the *Salmonella* chromosome (309 bp) with the 234 bp DNA fragment and the 480 bp DNA fragment encoding the structural portions of the *hfq*-like gene from *Staphylococcus aureus* and *Borrelia burgdorferi*, respectively. The construction was carried out as described in Materials and methods.

constructs (strains MA10741 and MA11042) showed that they carry no extra material or scars and that the hfq_{SA} and hfq_{BB} coding sequences are precisely positioned in replacement of the endogenous *hfq* coding sequence in the *Salmonella* chromosome (Fig. 1 and S1). We therefore expected that hfq_{SA} and hfq_{BB} would be expressed from the transcription/translation initiation signals of hfq_{STM}. To confirm this, strain derivatives carrying 3xFLAG epitope fusions to the 3′ ends of all three *hfq* genes were constructed, and expression of the carboxy-terminally tagged proteins assessed by Western blot analysis. As shown in Fig. 2, all three proteins accumulate at significant levels. Some differences in the intensity of the hybridization signals are apparent; however, the fact that they correlate with the molecular weights of the proteins suggest that they are intrinsic to the Western protocol (increasingly less quantitative with smaller molecules) and do not reflect a difference in expression rates. Overall, the data in Fig. 2 strongly suggest that hfq_{SA} and hfq_{BB} are expressed at levels closely comparable to that of hfq_{STM}.

3.2. Hfq_{SA} and Hfq_{BB} fail to replace Hfq_{STM} in sRNA-mediated regulation

Genes regulated directly or indirectly by small RNA constitute reporter systems for Hfq activity. Three representative loci were chosen for this analysis. The *chiP* gene of *Salmonella* encodes a chitoporin whose synthesis is repressed by a constitutively made

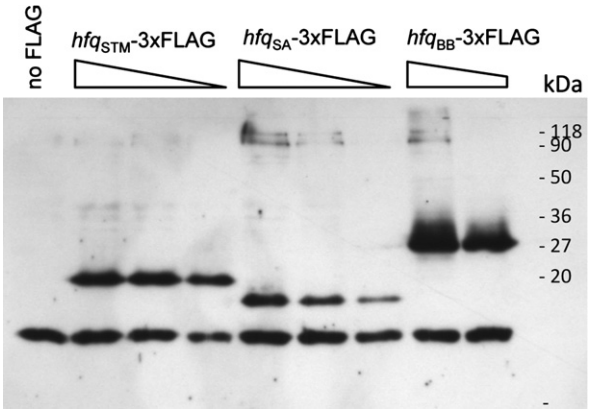

Fig. 2. Immunodetection of 3xFLAG-tagged Hfq_{STM}, Hfq_{SA} and Hfq_{BB} proteins in *Salmonella* extracts. Bacteria harvested from stationary cultures were lysed [37] and crude extracts were fractionated by electrophoresis in a 15% SDS-polyacrylamide gel. The gel was processed for the immunodetection of epitope-tagged proteins [37]. The strains used were (from left to right): MA9132 (wt, no FLAG), MA11054 (hfq_{STM}-3xFLAG), MA11055 (Δ*hfq*::hfq_{SA}-3xFLAG) and MA11056 (Δ*hfq*::hfq_{BB}-3xFLAG). The identity of the low molecular weight protein cross-reacting with the anti-FLAG antibodies is unknown.

sRNA, ChiX, which blocks *chiP* mRNA translation under most laboratory growth conditions. ChiX is the tightest Hfq-binding RNA known to date [42] and strongly dependent on Hfq-binding for stability [43]. Thus, a chromosomal translational *chiP-lacZ* fusion is expressed at very low level when Hfq is functional, but becomes derepressed nearly 40-fold in a strain deleted for *hfq* (Fig. 3A). As shown in Fig. 3A, strains carrying hfq_{SA} or hfq_{BB} in place of hfq_{STM} have β-galactosidase activities similar that of the *hfq* deleted strain. Consistent with these findings, Northern blot analysis reveals that ChiX sRNA is nearly undetectable in the hfq_{SA} or hfq_{BB}-expressing strains, like what observed in the absence of Hfq (Fig. 4). Failure of Hfq_{SA} or Hfq_{BB} to protect ChiX from degradation suggests that neither of the two proteins binds to this sRNA *in vivo*.

The second reporter system used in this study is the *eptB* gene, which encodes phosphoethanolamine transferase, an enzyme involved in lipopolysaccharide modification. A previous study identified *eptB* as one of the genes most dramatically upregulated in a Δ*hfq* mutant [18]. This results from the combined effect of two apparently independent mechanisms: activation of the σ^E regulon [18] and loss of repression by MgrR sRNA [44]. To examine whether Hfq_{SA} or Hfq_{BB} corrected these regulatory defects in any way, a translational *eptB-lacZ* fusion was introduced into in the hfq_{SA}- or hfq_{BB}-expressing strains and β-galactosidase activity measured. Again, the results in Fig. 3B show that the two strains are indistinguishable from the *hfq* null mutant in this test.

Both the above systems represent examples of negative regulation. We thus included a third reporter system in which Hfq activity is needed for gene activation. Due to the requirement of Hfq-dependent sRNAs for optimal translation of σ^S mRNA, genes controlled by σ^S are typically less expressed in the absence of Hfq [18]. One such gene encodes the KatE catalase. A further set of strains was therefore constructed carrying a *katE-lacZ* fusion in the hfq_{SA} or hfq_{BB} genetic background. As shown in Fig. 3C neither of the heterologous Hfq proteins can compensate for the loss of the endogenous Hfq as far as *katE-lacZ* expression is concerned.

3.3. Hfq_{SA} and Hfq_{BB} fail to protect sRNAs from degradation

As described above, ChiX sRNA is almost undetectable in the strains carrying hfq_{SA} or hfq_{BB} (Fig. 4). This analysis included additional representative sRNAs: GcvB, MicA, MicC and MicF. As shown in Fig. 4, the Northern hybridization patterns from the hfq_{SA}- and hfq_{BB}-carrying strains in all cases are indistinguishable from that of *hfq* deleted strain: MicC and MicF are both undetectable, while GcvB decreases significantly. In the case of MicA, the slight increase in sRNA levels is ascribable to σ^E activation since the *micA* gene is a member of the σ^E regulon [18,20]. The broadening of the MicA band, on the other hand, reflects the increased decay resulting from

Please cite this article in press as: T. Rochat, et al., Lack of interchangeability of Hfq-like proteins, Biochimie (2012), doi:10.1016/j.biochi.2012.01.016

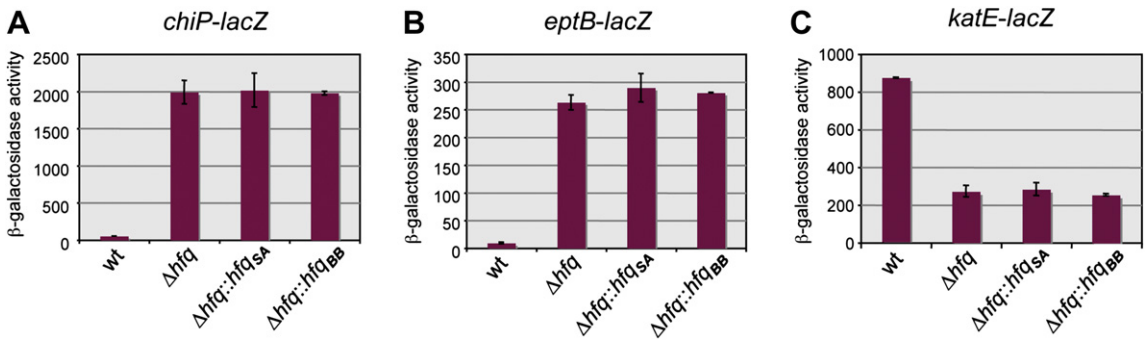

Fig. 3. Expression of translational *lacZ* fusions to chromosomal genes sensitive to Hfq inactivation in *Salmonella enterica*. β-galactosidase activity was measured in exponentially growing LB cultures ($OD_{600} \approx 0.3$) (A and B) or in stationary phase cultures ($OD_{600} \approx 2$) (C). Strain used were: A. MA9132, MA10744, MA10747 and MA11044; B. MA8028, MA8029, MA10746 and MA11043; C. MA8149, MA8679, MA11057 and MA11058 (see Table 1 for full genotypes).

the Hfq defect (see [18]). These findings are reminiscent of those by Sittka and co-workers who found *Methanococcus* Hfq incapable of reversing the chronic σ^E activation of a *Salmonella* strain lacking Hfq [26]. Altogether, the above data suggest that neither Hfq_{SA} nor Hfq_{BB} are capable of binding *Salmonella* sRNAs *in vivo*.

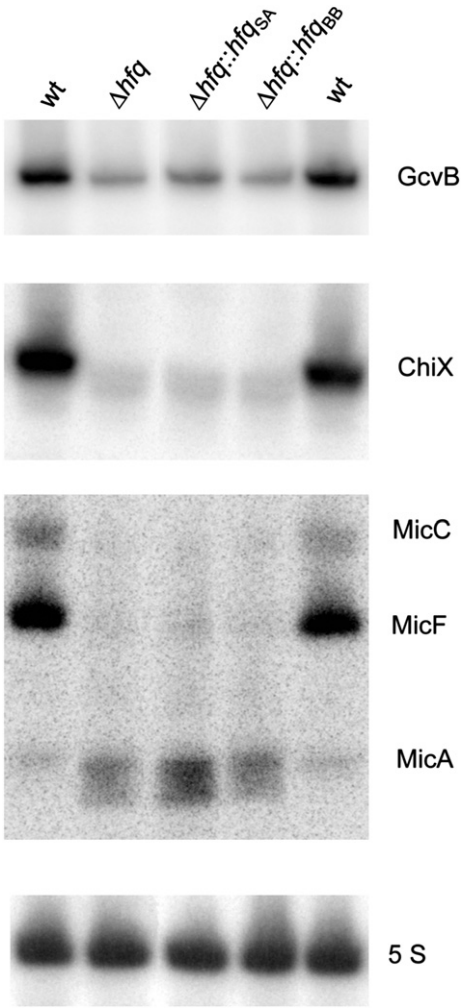

Fig. 4. Northern blot analysis of small RNAs. Total RNA extracted from exponentially growing cells ($OD_{600} = 0.35$) was fractionated on an 8% polyacrylamide-8 M urea gel and electrotransferred onto a Hybond-N^+ membrane. Blots were hybridized to 5′ end-labelled DNA oligonucleotide probes (Table 3). *Salmonella* strains used were: MA8028 (hfq_{STM} wt), MA8029 (Δhfq_{STM}::cat), MA10746 (Δhfq_{STM}::hfq_{SA}), MA11044 (Δhfq_{STM}::hfq_{BB}) and MA9132 (hfq_{STM} wt).

S. aureus Hfq is representative of bacterial Sm-like proteins and an inspirational reference in the small RNA field. Somewhat ironically, however, the function of this protein remains enigmatic. Although the protein was shown to bind RNAIII, a major RNA regulator in *S. aureus* [45], it does not appear to facilitate the RNAIII's interaction with any of the mRNA targets that have been looked at (reviewed in [46]). Data on the effects of *hfq* deletions on various aspects of *S. aureus* physiology are contradictory, possibly due to strain variability in the expression levels of *hfq* [30,33]. Considering that a possible redundancy in Hfq-like functions might underlie such variability in *S. aureus*, we chose to examine the functional status of Hfq_{SA} in *S. enterica*, where the role of the unique Hfq protein is well established. Our results showed that Hfq_{SA} does not participate in any of the sRNA-mediated regulatory mechanisms that were tested. Furthermore, we found no evidence of Hfq_{SA} binding to sRNAs *in vivo*. Altogether, these data indicate that Hfq_{SA} is functionally very different from its homologues in enteric bacteria. Such functional divergence might be in relation with the structural differences noted earlier on the distal face of the protein.

A second set data from this study shows that the Hfq-like protein from *B. burgdorferi* is also unable to replace Hfq_{STM} in sRNA-mediated regulation and sRNA stabilization. These findings are surprising as a recent publication reported Hfq_{BB} to complement the defect in *rpoS* mRNA regulation of an *E. coli hfq* mutant [25]. The discrepancy is difficult to reconcile. The authors of the above report performed their analysis using a plasmid-born hfq_{BB} gene; thus, in principle, the discrepancy could arise from gene dosage differences. Somehow, however, this explanation seems unlikely. An alternative explanation might lie in the nature of the *E. coli hfq* allele, *hfq*-1, used in the above study. The *hfq*-1 allele was originally constructed by inserting an Ω (KnR) cassette into the BclI site spanning positions 117–122 of the hfq_{EC} coding sequence [13]. Therefore, the mutant is expected to express a truncated Hfq fragment of about 40 amino acids, which would include the N-terminal α1 helix and two of the β strands of the Sm1 motif. Perhaps this fragment could somehow oligomerize with the Hfq_{BB} monomers to reconstitute a functional Hfq protein. Testing complementation in an *E. coli* strain in which the *hfq* gene is completely deleted would help remove this doubt.

3.4. Concluding remarks

The data presented here convincingly show that the Hfq proteins from *S. aureus* and *B. burgdorferi* are not interchangeable with their *Salmonella* counterpart as far as sRNA-mediated regulation is concerned. Clearly, more work is needed to elucidate the basis and biological rationale for the differences. Some of the strains

Please cite this article in press as: T. Rochat, et al., Lack of interchangeability of Hfq-like proteins, Biochimie (2012), doi:10.1016/j.biochi.2012.01.016

constructed in the course of this work might prove useful to this end. The 3xFLAG-tagged derivative of Hfq_{SA} and Hfq_{BB} could be used to test whether there exist RNAs that bind either of these proteins in *Salmonella* and possibly identify them [26]. As a complementary strategy, one could take advantage of the wide array of *Salmonella* genetic tools to search for genes whose expression might change in the presence or absence of Hfq_{SA} or Hfq_{BB}. These studies should help improve our understanding of the function, mode of action and ligand specificity of Sm-like proteins in bacteria.

Acknowledgements

This work was supported by a grant from the IFR 115, Genomes, Transcriptomes, Proteomes to NFB and a grant from the Agence Nationale pour la Recherche grant (ANR-2010-BLAN-1602-01 'Duplex-Omics') to PB. We thank Christine Pourcel (Orsay, France) for proving *B. burgdorferi* genomic DNA, and Satoko Yoshizawa and Dominique Fourmy (CGM, Gif-sur-Yvette) for helpful discussions.

Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.biochi.2012.01.016.

References

[1] G. Storz, J. Vogel, K.M. Wassarman, Regulation by small RNAs in bacteria: expanding frontiers, Mol. Cell 43 (2011) 880–891.
[2] M.T. Franze de Fernandez, L. Eoyang, J.T. August, Factor fraction required for the synthesis of bacteriophage Qβ-RNA, Nature 219 (1968) 588–590.
[3] R.G. Brennan, T.M. Link, Hfq structure, function and ligand binding, Curr. Opin. Microbiol. 10 (2007) 125–133.
[4] P. Valentin-Hansen, M. Eriksen, C. Udesen, The bacterial Sm-like protein Hfq: a key player in RNA transactions, Mol. Microbiol. 51 (2004) 1525–1533.
[5] J. Vogel, B.F. Luisi, Hfq and its constellation of RNA, Nat. Rev. Microbiol. 9 (2011) 578–589.
[6] H. Aiba, Mechanism of RNA silencing by Hfq-binding small RNAs, Curr. Opin. Microbiol. 10 (2007) 134–139.
[7] T.M. Link, P. Valentin-Hansen, R.G. Brennan, Structure of *Escherichia coli* Hfq bound to polyriboadenylate RNA, Proc. Natl. Acad. Sci. U S A 106 (2009) 19292–19297.
[8] C. Sauter, J. Basquin, D. Suck, Sm-like proteins in Eubacteria: the crystal structure of the Hfq protein from *Escherichia coli*, Nucleic Acids Res. 31 (2003) 4091–4098.
[9] M.A. Schumacher, R.F. Pearson, T. Moller, P. Valentin-Hansen, R.G. Brennan, Structures of the pleiotropic translational regulator Hfq and an Hfq-RNA complex: a bacterial Sm-like protein, EMBO J. 21 (2002) 3546–3556.
[10] P.J. Mikulecky, M.K. Kaw, C.C. Brescia, J.C. Takach, D.D. Sledjeski, A.L. Feig, *Escherichia coli* Hfq has distinct interaction surfaces for DsrA, *rpoS* and poly(A) RNAs, Nat. Struct. Mol. Biol. 11 (2004) 1206–1214.
[11] A.S. Olsen, J. Moller-Jensen, R.G. Brennan, P. Valentin-Hansen, C-terminally truncated derivatives of *Escherichia coli* Hfq are proficient in riboregulation, J. Mol. Biol. 404 (2010) 173–182.
[12] B. Vecerek, L. Rajkowitsch, E. Sonnleitner, R. Schroeder, U. Blasi, The C-terminal domain of *Escherichia coli* Hfq is required for regulation, Nucleic Acids Res. 36 (2008) 133–143.
[13] H.C. Tsui, H.C. Leung, M.E. Winkler, Characterization of broadly pleiotropic phenotypes caused by an *hfq* insertion mutation in *Escherichia coli* K-12, Mol. Microbiol. 13 (1994) 35–49.
[14] F. Repoila, N. Majdalani, S. Gottesman, Small non-coding RNAs, co-ordinators of adaptation processes in *Escherichia coli*: the RpoS paradigm, Mol. Microbiol. 48 (2003) 855–861.
[15] L. Bossi, D. Maloriol, N. Figueroa-Bossi, Porin biogenesis activates the σ^E response in *Salmonella hfq* mutants, Biochimie 90 (2008) 1539–1544.
[16] Y. Ding, B.M. Davis, M.K. Waldor, Hfq is essential for *Vibrio cholerae* virulence and downregulates sigma expression, Mol. Microbiol. 53 (2004) 345–354.
[17] V. Douchin, C. Bohn, P. Bouloc, Down-regulation of porins by a small RNA bypasses the essentiality of the regulated intramembrane proteolysis protease RseP in *Escherichia coli*, J. Biol. Chem. 281 (2006) 12253–12259.
[18] N. Figueroa-Bossi, S. Lemire, D. Maloriol, R. Balbontín, J. Casadesús, L. Bossi, Loss of Hfq activates the σ^E-dependent envelope stress response in *Salmonella enterica*, Mol. Microbiol. 62 (2006) 838–852.
[19] E. Guisbert, V.A. Rhodius, N. Ahuja, E. Witkin, C.A. Gross, Hfq modulates the σ^E-mediated envelope stress response and the σ^{32}-mediated cytoplasmic stress response in *Escherichia coli*, J. Bacteriol. 189 (2007) 1963–1973.
[20] K. Papenfort, V. Pfeiffer, F. Mika, S. Lucchini, J.C. Hinton, J. Vogel, σ^E-dependent small RNAs of *Salmonella* respond to membrane stress by accelerating global *omp* mRNA decay, Mol. Microbiol. 62 (2006) 1674–1688.
[21] X. Sun, I. Zhulin, R.M. Wartell, Predicted structure and phyletic distribution of the RNA-binding protein Hfq, Nucleic Acids Res. 30 (2002) 3662–3671.
[22] Y. Chao, J. Vogel, The role of Hfq in bacterial pathogens, Curr. Opin. Microbiol. 13 (2010) 24–33.
[23] E. Sonnleitner, I. Moll, U. Blasi, Functional replacement of the Escherichia coli *hfq* gene by the homologue of *Pseudomonas aeruginosa*, Microbiology 148 (2002) 883–891.
[24] A.S. Attia, J.L. Sedillo, W. Wang, W. Liu, C.A. Brautigam, W. Winkler, E.J. Hansen, *Moraxella catarrhalis* expresses an unusual Hfq protein, Infect. Immun. 76 (2008) 2520–2530.
[25] M.C. Lybecker, C.A. Abel, A.L. Feig, D.S. Samuels, Identification and function of the RNA chaperone Hfq in the Lyme disease spirochete *Borrelia burgdorferi*, Mol. Microbiol. 78 (2010) 622–635.
[26] A. Sittka, C.M. Sharma, K. Rolle, J. Vogel, 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 (2009) 266–275.
[27] A. Gaballa, H. Antelmann, C. Aguilar, S.K. Khakh, K.B. Song, G.T. Smaldone, J.D. Helmann, The *Bacillus subtilis* iron-sparing response is mediated by a Fur-regulated small RNA and three small, basic proteins, Proc. Natl. Acad. Sci. U S A 105 (2008) 11927–11932.
[28] N. Heidrich, A. Chinali, U. Gerth, S. Brantl, The small untranslated RNA SR1 from the *Bacillus subtilis* genome is involved in the regulation of arginine catabolism, Mol. Microbiol. 62 (2006) 520–536.
[29] J.M. Silvaggi, J.B. Perkins, R. Losick, Small untranslated RNA antitoxin in *Bacillus subtilis*, J. Bacteriol. 187 (2005) 6641–6650.
[30] C. Bohn, C. Rigoulay, P. Bouloc, No detectable effect of RNA-binding protein Hfq absence in *Staphylococcus aureus*, BMC Microbiol. 7 (2007) 10.
[31] E. Geisinger, R.P. Adhikari, R. Jin, H.F. Ross, R.P. Novick, Inhibition of *rot* translation by RNAIII, a key feature of *agr* function, Mol. Microbiol. 61 (2006) 1038–1048.
[32] B. Felden, F. Vandenesch, P. Bouloc, P. Romby, The *Staphylococcus aureus* RNome and its commitment to virulence, PLoS Pathog. 7 (2011) e1002006.
[33] Y. Liu, N. Wu, J. Dong, Y. Gao, X. Zhang, C. Mu, N. Shao, G. Yang, Hfq is a global regulator that controls the pathogenicity of *Staphylococcus aureus*, PLoS One 5 (2010).
[34] N. Figueroa-Bossi, E. Coissac, P. Netter, L. Bossi, Unsuspected prophage-like elements in *Salmonella typhimurium*, Mol. Microbiol. 25 (1997) 161–173.
[35] H. Schmieger, Phage P22-mutants with increased or decreased transduction abilities, Mol. Gen. Genet. 119 (1972) 75–88.
[36] K.A. Datsenko, B.L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products, Proc. Natl. Acad. Sci. U S A 97 (2000) 6640–6645.
[37] S. Uzzau, N. Figueroa-Bossi, S. Rubino, L. Bossi, Epitope tagging of chromosomal genes in *Salmonella*, Proc. Natl. Acad. Sci. U S A 98 (2001) 15264–15269.
[38] J.E. Karlinsey, λ-Red genetic engineering in Salmonella enterica serovar Typhimurium, Methods Enzymol. 421 (2007) 199–209.
[39] B.R. Bochner, H.C. Huang, G.L. Schieven, B.N. Ames, Positive selection for loss of tetracycline resistance, J. Bacteriol. 143 (1980) 926–933.
[40] L. Bossi, N. Figueroa-Bossi, A small RNA downregulates LamB maltoporin in *Salmonella*, Mol. Microbiol. 65 (2007) 799–810.
[41] J.H. Miller, A Short Course in Bacterial Genetics. A Laboratory Manual and Handbook for Escherichia Coli and Related Bacteria, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1992.
[42] K. Moon, S. Gottesman, Competition among Hfq-binding small RNAs in Escherichia coli, Mol. Microbiol. 82 (2011) 1545–1562.
[43] N. Figueroa-Bossi, M. Valentini, L. Malleret, F. Fiorini, L. Bossi, Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target, Genes Dev. 23 (2009) 2004–2015.
[44] K. Moon, S. Gottesman, A PhoQ/P-regulated small RNA regulates sensitivity of *Escherichia coli* to antimicrobial peptides, Mol. Microbiol. 74 (2009) 1314–1330.
[45] E. Huntzinger, S. Boisset, C. Saveanu, Y. Benito, T. Geissmann, A. Namane, G. Lina, J. Etienne, B. Ehresmann, C. Ehresmann, A. Jacquier, F. Vandenesch, P. Romby, *Staphylococcus aureus* RNAIII and the endoribonuclease III coordinately regulate *spa* gene expression, EMBO J. 24 (2005) 824–835.
[46] A. Jousselin, L. Metzinger, B. Felden, On the facultative requirement of the bacterial RNA chaperone, Hfq, Trends Microbiol. 17 (2009) 399–405.