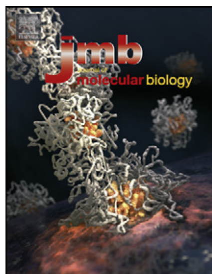

# Arginine Patch Predicts the RNA Annealing Activity of Hfq from Gram-Negative and Gram-Positive Bacteria

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http://dx.doi.org/10.1016/j.jmb.2016.03.027
***Edited by Anna Pyle***

## Abstract

The Sm-protein Hfq facilitates interactions between small non-coding RNA (sRNA) and target mRNAs. In enteric Gram-negative bacteria, Hfq is required for sRNA regulation, and *hfq* deletion results in stress intolerance and reduced virulence. By contrast, the role of Hfq in Gram-positive is less established and varies among species. The RNA binding and RNA annealing activity of Hfq from *Escherichia coli*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus subtilis,* and *Staphylococcus aureus* were compared using minimal RNAs and fluorescence spectroscopy. The results show that RNA annealing activity increases with the number of arginines in a semi-conserved patch on the rim of the Hfq hexamer and correlates with the previously reported requirement for Hfq in sRNA regulation. Thus, the amino acid sequence of the arginine patch can predict the chaperone function of Hfq in sRNA regulation in different organisms.
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Hfq is an abundant bacterial member of the Sm-superfamily that was originally discovered as an essential host factor for the replication of *Escherichia coli* bacteriophage Qβ [1,2]. More recently, the role of Hfq in small RNA (sRNA)-mediated gene regulation has received considerable attention [3]. sRNAs regulate diverse physiological behaviors such as stationary phase, stress responses, sugar metabolism, iron utilization, and quorum sensing [4,5,6].

The Sm core of Hfq forms a ring-shaped homohexamer that binds the U-rich terminator at the 3′ end of most sRNAs and stabilizes the sRNA against turnover [7,8]. In most Gram-negative bacteria, the opposite (distal) face binds single-stranded A-rich RNA sequences found in mRNA targets and some sRNAs [9,10,11,12]. Importantly, complementary sequences in the sRNA and mRNA target also interact with an arginine patch (R16, R17, and R19) on the rim of the *E. coli* Hfq hexamer [13,14,15,16,17] that is essential for Hfq's RNA annealing activity [15]. *In vitro* annealing assays showed that *E. coli* Hfq accelerates sRNA–mRNA base pairing 30 to 100 times by nucleating the double helix between two complementary RNA strands [18,19]. This annealing activity is eliminated when all three arginines are replaced with alanine, and even an arginine to lysine substitution reduces *E. coli* Hfq's chaperone activity [15].

Although Hfq is needed for sRNA regulation in Gram-negative bacteria such as *E. coli* and *Salmonella* [20], the requirement for Hfq in post-transcriptional regulation by Gram-positive bacteria remains ambiguous [21]. Hfq contributes to stress tolerance and pathogenicity in *Listeria monocytogenes* [22], *Clostridium difficile* [23], and other Gram-positive bacteria [24]. By contrast, deletion of Hfq from *Bacillus subtilis* and *Staphylococcus aureus* has almost no detectable phenotype [25,26].

We noticed that the reported role of Hfq in sRNA-mediated gene regulation correlates with variations in the amino acid sequence of the basic patch on the Hfq rim [15]. Although the potent *E. coli* Hfq chaperone has three arginines between residues 16–19 (RRER), the *B. subtilis* protein has only one arginine (RKEN) and the *S. aureus* protein has none (KANQ) (Fig. 1). Here, we use a well-established fluorescent RNA annealing assay to measure the chaperone activity of Hfq homologs from two Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*) and three Gram-positive bacteria (*L. monocytogenes*, *B. subtilis,* and *S. aureus*). We show that the RNA annealing activity increases with the number of arginine residues on the rim, with Gram-positive Hfq proteins having little or no activity in our assay. Thus, the amino acid sequence of the

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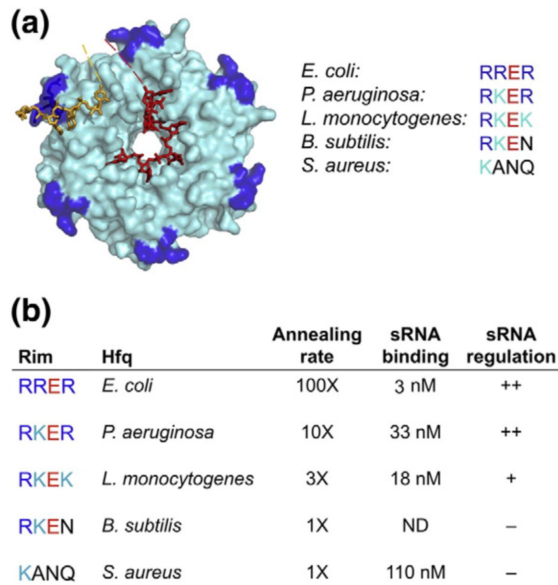


| Rim | Hfq | Annealing rate | sRNA binding | sRNA regulation |
|---|---|---|---|---|
| RRER | *E. coli* | 100X | 3 nM | ++ |
| RKER | *P. aeruginosa* | 10X | 33 nM | ++ |
| RKEK | *L. monocytogenes* | 3X | 18 nM | + |
| RKEN | *B. subtilis* | 1X | ND | – |
| KANQ | *S. aureus* | 1X | 110 nM | – |

**Fig 1.** Basic patch on the rim of Hfq. (a) *E. coli* Hfq (*1HK9*) rendered as cyan surface, superimposed with the structure of RydC sRNA in complex with Hfq (*4V2S*). Red sticks, 3′ $U_6$ in the proximal pore; yellow sticks, internal 5′ CUUC bound to the rim of Hfq. The remaining RydC residues are omitted for clarity. Blue surface, arginine patch with R16, R17, and R19 side chains shown for one subunit. The rim sequences of Hfq proteins used in this study are shown on the right. (b) Summary of Hfq activity from different organisms. The RNA annealing rates relative to no Hfq and sRNA binding are from this work; sRNA regulation is based on published work, as described in the text.

arginine patch predicts Hfq's chaperone activity and the degree to which Hfq facilitates interactions between sRNA and mRNA pairs in different bacteria.

## RNA annealing activity of Hfq from different bacteria

An alignment of Hfq sequences from different bacteria revealed a partial conservation of amino acid residues that lie on the outer rim of the Hfq hexamer [15]. Nearly all Hfq sequences contain an arginine at position 16 (*E. coli* numbering) on the proximal side of the rim. Position 17 is usually R or K, position 18 is neutral polar or acidic, and position 19, which lies closest to the distal face, is the most variable [15]. In order to understand how this

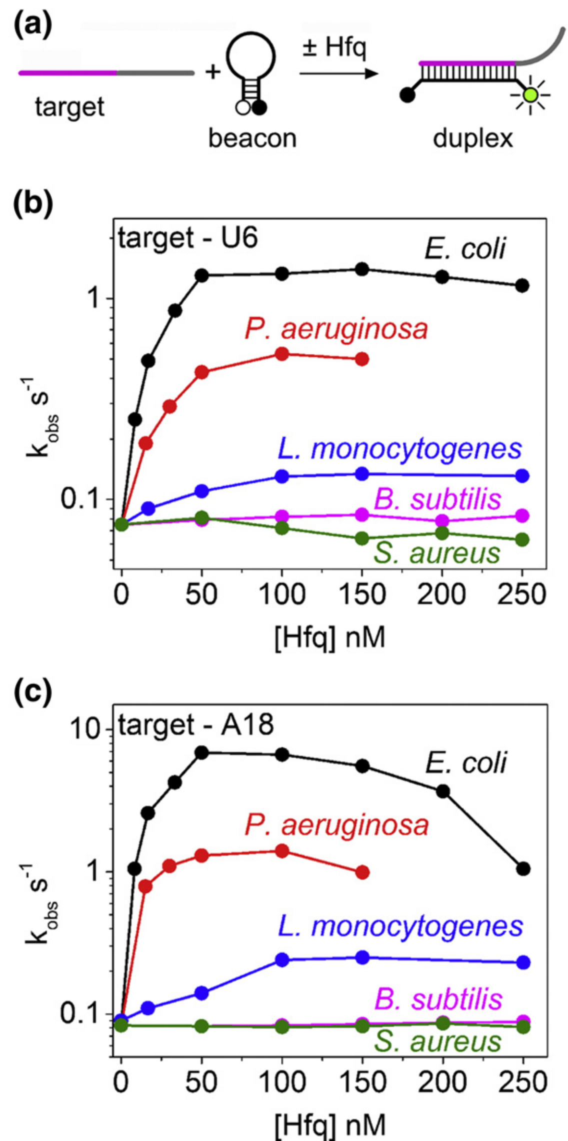


**Fig 2**. RNA annealing activity depends on the rim sequence. *E. coli*, *B. subtilis, and S. aureus* Hfqs were purified using a Ni-affinity column, as described previously [29]. *P. aeruginosa* and *L. monocytogenes* Hfqs were purified using the IMPACT-CN system [30]. All proteins were further purified using a cation exchange column (GE) [29] and verified by SDS-PAGE. $A_{260}/A_{280}$ ~ 0.7 for all of the preparations. (a) Molecular beacon assay for RNA annealing. The loop of the molecular beacon (5′FAM-GGUCCC**CCACUCGACUCAC-CAC** CGGACC-3′DABCYL) is complementary to target-U6 (5′ **GUGGUCAGUCGAGUGG**$U_6$) and target-A18 (5′ **GUGGUCAGUCGAGUGG**$A_{18}$). The increase in fluorescence intensity due to base pairing between molecular beacon and target RNAs was monitored by an Applied Photophysics SX 18MV stopped-flow spectrometer, as described previously [27]. 50 nM molecular beacon and 100 nM target RNAs were rapidly mixed in 0–250 nM Hfq in TNK buffer [10 mM Tris–HCl (pH 7.5); 50 mM NaCl and 50 mM KCl] at 30 °C. Individual kinetic traces were fitted to a double exponential rate equation (18). (b) Rate constants for annealing target-U6 at different concentrations of Hfq hexamer. The rate constants are the average of five technical replicates with standard deviations less than 5%. (c) Rate constants of annealing target-A18 *versus* Hfq concentration, as in (b).

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