Published in final edited form as:

Biochim Biophys Acta. 2010 August ; 1799(8): 588–596. doi:10.1016/j.bbagr.2010.06.007.

***E. coli* DNA associated with isolated Hfq interacts with Hfq's distal surface and C-terminal domain**

Taylor B. Updegrove^a, John J. Correia^b, Roberto Galletto^{c,1}, Wlodzimierz Bujalowski^c, and Roger M. Wartell^{a,*}

^a School of Biology and Parker H. Petit Institute for Bioscience and Bioengineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

^b Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216, USA

^c Department of Biochemistry, University of Texas Medical Branch at Galveston, Galveston, TX 77555, USA

Abstract

The RNA-binding protein Hfq has been studied extensively for its function as a modulator of gene expression at the post-transcriptional level. While most Hfq studies have focused on the protein's interaction with sRNAs and mRNAs, Hfq binding to DNA has been observed but is less explored. During the isolation of Hfq from *Escherichia coli*, we found genomic DNA fragments associated with the protein after multiple steps of purification. Sequences of 41 amplified segments from the DNA fragments associated with Hfq were determined. A large fraction of the DNA segments were predicted to have significant helical axis curvature and were from genes associated with membrane proteins, characteristics unexpected for non-specific binding. Analysis by analytical ultracentrifugation indicated that rA₁₈ binding to Hfq disrupts Hfq-DNA interactions. The latter observation suggests Hfq binding to DNA involves its distal surface. This was supported by a gel mobility shift assay that showed single amino acid mutations on the distal surface of Hfq inhibited Hfq binding to duplex DNA, while six of seven mutations on the proximal surface and outer circumference of the hexamer did not prevent Hfq binding. Two mutated Hfq which have portions of their C-terminal domain removed also failed to bind to DNA. The apparent K_d for binding wild type Hfq to several duplex DNA was estimated from a gel mobility shift assay to be ~400 nM.

Keywords

Hfq protein; Hfq-DNA binding

1. Introduction

The Hfq protein of *Escherichia coli* (also known as HF-1) is an RNA-binding protein and a key factor in post-transcriptional gene regulation [1–6]. The pleiotropic phenotypic effects that results from inactivating the Hfq gene in *E. coli* [7] and other gram negative bacterial species has been linked to Hfq's role in facilitating the interaction of small regulatory RNAs (sRNAs) with messenger RNAs (mRNAs) and as a modulator of sRNA and mRNA stability. Hfq's role in regulating mRNA translation by sRNAs is an important feature of

bacterial adaptation to stress and the establishment of virulence [8–12]. Many studies of Hfq have been directed towards understanding its interaction with sRNAs and mRNAs in both *in vitro* and *in vivo* contexts. However, several studies on proteins associated with the *E. coli* nucleoid DNA suggested that Hfq binding to DNA may also have a functional role [13,14].

Hfq was among the ten most prevalent proteins associated with nucleoid DNA isolated from *E. coli* [14]. In exponentially growing cells, it was the third most prevalent of the ten proteins. *In situ* immunofluorescence studies indicated that most Hfq appears to be in the cytoplasm (80–90%), however a portion of this protein was found in the DNA nucleoid region of the cell (10–20%) [13,15]. Recent electron microscopy studies have confirmed the presence of Hfq in the cytoplasm and nucleoid and demonstrated that Hfq is also localized close to the inner membrane [16]. Plasmid DNAs grown in *E. coli* were shown to bind Hfq *in vivo* and *in vitro* [17]. Apparent equilibrium dissociation constants (K_d) for Hfq binding to two 60-bp DNA duplexes were reported as 125 and 250 nM [14]. Greater affinity was exhibited for the curved DNA than for the mixed sequence DNA. Although the above range of affinities is weaker than Hfq affinity for sRNAs (K_d ~20–50 nM), it is tight enough to suggest that Hfq binding to DNA may play a functional role *in vivo*, particularly in light of Hfq's μ M-level cellular abundance [1].

In the current work, we have characterized *E. coli* genomic DNA fragments found associated with Hfq purified from lysed cells and investigated the nature of the Hfq–DNA interaction. Several lines of evidence indicate that Hfq binding to DNA involves the protein's distal surface and C-terminal domain. The sequences of amplified segments of the genomic DNA exhibit several interesting characteristics. Over half are predicted to have helical axis curvature and are predominantly from genes coding for membrane proteins.

2. Materials and methods

2.1. Purification and characterization of wt Hfq and mutant Hfq

The Impact-CN intein system (New England Biolabs) was used to produce and purify Hfq proteins as previously described [18]. The *E. coli* *hfq* gene was cloned into the pTYB11 plasmid to create the expression plasmid pTYB11-wt Hfq. Hfq was expressed from this plasmid in *E. coli* strain ER2566 using the recommendations of the manufacturer. Cells were lysed using a French press in 0.5 M NaCl, 20 mM Tris (pH 8.3), 0.1 mM EDTA, 0.1% Triton X100, and 5% glycerol. The cell lysate was centrifuged and the supernatant loaded onto a chitin column. The column was extensively washed (15 to 20 bed volumes) with the wash buffer that consisted of 20 mM Tris (pH 8.3) and 0.5 or 1.0 M NaCl with or without 0.1% Triton X100 (all variations gave similar outcomes). The column was then incubated with 0.5 M NaCl and 20 mM Tris buffer plus 40 mM dithiothreitol. Eluted protein was concentrated and buffer-exchanged to 0.5 M NaCl and 20 mM Tris at pH 8.3 using 30 kD MWCO centrifugation filtration units. The protein preparation at this stage is referred to as Hfq-NA. Hfq was further purified by either a DEAE column or more commonly by a nuclease treatment to remove 250–260 nm absorbing material.

The nuclease treatment of Hfq-NA preparations was carried out by adding 7.5 U of micrococcal nuclease (Worthington Biochemical Corporation) to 1 ml of 0.2–0.4 OD_{274nm} units of the protein sample in a solvent of 0.2 M NaCl, 20 mM Tris (pH 8.3) and 5 mM CaCl₂. We note that micrococcal nuclease activity is absolutely dependent on Ca²⁺. Reactions were incubated at 37 °C for 1 h and terminated by adding 10 μ l of 0.5 M Na₂EDTA. Reactions were then extensively buffer exchanged with 0.5 M NaCl and 20 mM Tris (pH 8.3) and their volumes reduced to ~1 ml using a 15 ml 30 kD centrifugation filter. This approach was more consistent than a DEAE column in giving a high A₂₇₅/A₂₅₀ absorbance ratio (Fig. 1A).

Plasmids containing mutant *hfq* genes were generated from pTYB11-wtHfq using the QuikChange Mutagenesis Kit from Stratagene Inc [19]. In addition to the previously described mutations F42A, F39A, Q8A, R16A, K31A, and Y25A [18], *hfq* genes with single residue mutations R19A, R17A, and F11A were constructed and their proteins expressed. Two additional mutant *hfq* genes were constructed by creating stop codons at residues 76 and 66, respectively. These plasmids yielded truncated Hfq designated Hfq-65 and Hfq-75. The wt Hfq and mutant Hfqs were characterized for purity by SDS-PAGE and UV spectroscopy [18].

2.2. Analytical ultracentrifugation

Sedimentation velocity studies were performed using a Beckman Optima XLA analytical ultracentrifuge equipped with absorbance optics and an An60 Ti rotor at 19.7 °C. Temperature was calibrated as described previously [20]. Velocity data were typically collected at appropriate speeds using 274 nm to monitor Hfq and Hfq-NA, and 495 nm when FAM-A₁₈ was added. Spacing of 0.002 cm was employed with one flash at each point in a continuous-scan mode. All experiments were initially analyzed with Sedfit to produce $c(s)$ distributions [21] and with DCDT² to produce $g(s)$ distributions and weight average S values [22]. Direct boundary fitting of velocity data to discrete models were also performed with the program Sedanal [23]. Analysis with Sedanal requires input of MW, extinction coefficients, and density increments (typically estimated from $1-\bar{v} \cdot \rho$ values). The buffer solution density for 0.5 M NaCl and 20 mM Tris (8.3) was estimated in Sednterp to be 1.01920 gm/ml at 19.7 °C. The $\bar{v}$ of Hfq was estimated with Sednterp [24] to be 0.7248. The $\bar{v}$ of FAM-A₁₈ is assumed to be 0.55. The extinction coefficient for FAM-A₁₈ at 495 nm is 75,000 M⁻¹ cm⁻¹; the extinction coefficient of Hfq at 274 nm is 4350 M⁻¹ cm⁻¹ [18]. Parameter uncertainty is calculated with an Fstat routine within Sedanal at the 95% confidence interval and reported in a < , > format.

Sedimentation equilibrium studies were carried out on Hfq-NA at 1.75, 3.5, and 7 μM. The solvent for most studies was 0.5 M NaCl and 20 mM Tris (8.3). Employing a buffer of 0.2 M NaCl and 20 mM Tris (8.3) gave similar results. Samples were centrifuged at 12,000 rpm and 16,000 rpm at a temperature of 19.7 °C in six channel double sector cells. Data were collected at 274 nm. Equilibrium at each speed was judged with the software utility WinMATCH (<http://www.biotech.uconn.edu/auf/?i=aufftp>). This program makes a least-square comparison of successive scans to establish that equilibrium has been achieved. Values for density, $\bar{v}$ and extinction coefficients were as described above for sedimentation velocity measurements. Non-linear least squares fit of sedimentation equilibrium profiles to a model of two independent noninteracting components gave a much better fit than to a single species model (Fig. 2B). The six data sets from three concentrations and two speeds were best fit to a two species model using Sedanal. Molecular weight uncertainty is calculated with Fstat as described above.

2.3. Characterization of nucleic acid in Hfq-NA

The nucleic acid associated with Hfq in Hfq-NA was characterized by examining the aqueous phase after phenol-chloroform extraction. 30 μl of ~10 μM Hfq-NA was phenol-chloroform extracted and then analyzed on a 1% agarose gel, staining with ethidium bromide. The effect of RNase A on Hfq-NA was examined by adding 1 μg of RNase A (Promega) to 30 μl of Hfq-NA and incubating for 30 min at 37 °C prior to phenol-chloroform extraction. The influence of DNase on Hfq-NA was examined by adding 6 units of DNase I (Promega) and incubating for 30 min at 37 °C. Control experiments using either yeast RNA or *E coli* genomic DNA verified the effectiveness of these nucleases. The aqueous phases from the phenol extractions were loaded onto a 1% agarose gel along with Hfq-NA not treated with nuclease.

2.4. Isolation and cloning of DNA associated with Hfq

4 µg of proteinase K (Sigma Aldrich) was added to 80 µl of ~0.8 OD₂₇₄ non-nuclease treated Hfq-NA and incubated for 2 h at 50 °C. 10 µl of 10× gel loading buffer (0.2 M NaCl, 20 mM Tris (pH 8.3), 40% glycerol and 0.02% (wt/vol) bromophenol blue) was then added to the sample and 45 µl loaded directly into two lanes of a 1% agarose gel. Following staining of the gel with ethidium bromide, the smeared DNA fragments were excised from the gel under 350 nm UV light and the DNA purified using IsoPure™ DNA Purification Kit (Denville Scientific). PCR amplification of ~20 ng of this DNA or *E. coli* genomic DNA as a control was carried out with tagged random primers [25]. The tagged random primer employed was 5'-GGTAATC GGATCCAAGCNNNNNN-3'. The six underlined bases denote the *Bam*HI recognition site. After initial 1 min incubation at 95 °C, samples were exposed to 45 cycles of 96 °C for 30 s, 30 °C for 1 min, 40 °C for 1 min, and 72 °C for 2 min, followed by a final incubation at 72 °C for 5 min. PCR products were purified from primers and reaction buffer using QIAquick® PCR purification kit (Qiagen) and quantified by UV absorbance. A second PCR reaction used the tagged primer 5'-GGTAATCGGATCCAAGC-3' and 20 ng from the first PCR reaction with conditions of 95 °C for 2 min, followed by 35 cycles of 95 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min, and a final incubation at 72 °C for 5 min. Product from the second PCR reaction was digested with *Bam*HI and cloned into *Bam*HI digested pUC19 plasmid treated with Antarctic Phosphatase (New England Biolabs). Blue-white screening was used to isolate transformants with inserts. The inserts of purified plasmids were sequenced and searched against the Microbial Genome database using BLAST at the NCBI website (http://www.ncbi.nlm.nih.gov/cig-bin/Entrez/genome_table.cgi). 20 nt primers were used to amplify several of the cloned DNA segments in Table 1 for Hfq binding and gel mobility studies (Table S1). Following PCR reactions, the products were purified from primers, enzymes and buffer using QIAquick PCR Purification Kit (Qiagen) and analyzed on a 1% agarose gel to assess purity and later on a 10% polyacrylamide gel to assess their helical axis curvature [26]. DNAs used for binding studies were ³²P labeled at the 5' ends using standard protocols and purified from [γ-³²P]ATP and buffer exchanged using centrifugal filtration with a 10kD MWCO filter.

2.5. Electrophoretic gel mobility shift assay

Binding reactions were carried out in a solution composed of 0.2 M NaCl, 20 mM Tris-HCl (pH 8.0), 0.05% bromophenol blue and 5% glycerol in a 15-µl volume. Hfq was mixed with nucleic acids and maintained at room temperature for at least 30 min prior to loading and running on the gel. The gels were 5% polyacrylamide [29:1 (w/w) acrylamide/bisacrylamide] with 3% glycerol in 0.5 X TBE. Electrophoresis was conducted at room temperature at 80–100 V. Gels were either stained after the run with SYBR® Gold nucleic acid stain (Invitrogen) or if ³²P-labeled DNA was employed were imaged using a Fujifilm Image Reader FLA-3000. The gel images were analyzed using the Fujifilm Multi Gauge Imaging Software V3.0. The sRNAs DsrA and RprA employed in this assay were transcribed and purified from previously constructed plasmids [27] using a MEGAscript-T7 kit (Ambion Inc).

2.6. DNA sequence analysis

The DNA fragments associated with Hfq were examined for their propensity for helical axis curvature by calculating bendability/curvature propensity plots for their sequences using the BEND.IT server (http://hydra.icgeb.trieste.it/dna/bend_it.html). The DNase I based bendability parameters [28] and the consensus bendability parameters [29] were employed. Curvature scores were calculated using the default window size of 31 bp and are given in degrees/helical turn.

A search for conserved DNA sequence motifs among the DNA fragments isolated from Hfq-NA was made using the Gibbs Centroid Sampler [30]. This method utilizes information from the full ensemble of possible solutions and has been shown to improve motif finding compared to single optimal alignment of motif sites. The probability that x DNA fragments in a sample of n cloned DNA fragments are membrane protein genes was estimated using the hypergeometric probability distribution (<http://stattrek.com/Lesson2/Hypergeometric.aspx>). This probability distribution predicts the probability of sampling n items without replacement and finding x of the items with a specific property when the total population of N items has k items with the specific property. Assumptions made in applying this distribution to the cloned DNA fragments are discussed in Results.

3. Results

Hfq protein produced by the pTYB11-wtHfq expression plasmid in *E. coli* and purified using affinity column chromatography and centrifugal filtration (see Materials and methods) consistently exhibited UV spectra that suggested the presence of a small amount of contaminating nucleic acid (Fig. 1A). The peak of the UV spectrum was at the expected value of ~274 nm, but the absorbance ratio A_{274}/A_{250} of ~1.3 was lower than predicted for a protein with Hfq's amino acid composition (SEDNTERP ver.1.09, <http://www.jphilo.mailway.com> [24]). Although UV absorbing contamination could be a byproduct of the isolation procedure, the observation by other researchers of similar spectral characteristics using different methods of Hfq isolation [13,31] suggested nucleic acid contamination may be a common occurrence in Hfq purification.

A micrococcal nuclease treatment followed by centrifugal filtration was added as a final step of Hfq purification. This step resulted in Hfq spectra with A_{274}/A_{250} ratios ≥ 1.8 (Fig. 1A) supporting the hypothesis that nucleic acids had been removed. Centrifugal filtration without the nuclease treatment was not effective. We refer to Hfq samples prior to removal of the contaminating nucleic acid as Hfq-NA. Changes in cell growth temperature or method of cell lysis (sonication) did not alter the UV spectral characterization or analytical ultracentrifugation characterization described below.

3.1. Analytical ultracentrifugation analysis

Sedimentation velocity experiments monitored at 274 nm showed a significant difference between purified Hfq and Hfq-NA (Fig. 1B). The normalized $g(s)$ distribution of purified Hfq exhibited a single major species with a sedimentation coefficient (s) of $s=3.42\text{ S} <3.41, 3.44>$. Analysis of the data using Sedanal [23] yielded a molecular weight of 64,815 D $<59733, 70301>$. This value is slightly lower than the expected value of 66,998 D for the hexamer and is consistent with the hexamer as the dominant Hfq species. The slightly lower than expected value can be explained by uncertainty in the partial specific volume employed.

The normalized $g(s)$ distribution of Hfq-NA showed two species. The dominant species has the same sedimentation coefficient as Hfq₆. The second component has a sedimentation coefficient of $s_2 = 7.42\text{ S} <7.31, 7.53>$. A similar sedimentation velocity profile has been previously observed for Hfq [32]. The sedimentation coefficient distributions of Hfq and Hfq-NA were independent of loading concentrations from 3 to ~8 μM Hfq (moles hexamer). The faster moving species in the $g(s)$ distribution of Hfq-NA could be a multimer of Hfq₆ [32], but given the different UV absorbance spectra and sedimentation profiles of Hfq-NA and Hfq, a nucleic acid or nucleic acid-Hfq complex seemed more likely. Experiments described below indicate this species is a Hfq-nucleic acid complex.

Previous work has shown that rA_n with $n > 18$ bind Hfq₆ with high affinity ($K_d \sim 10$ nM) and interacts with Hfq₆'s distal surface [18,33]. Fig. 2A shows that the addition of 3 μ M FAM-labeled rA_{18} to 3 μ M Hfq-NA disrupts the 7.4 S species and yields a new macromolecule with a sedimentation coefficient of 3.8 S monitored at 495 nm or 274 nm. FAM- rA_{18} alone has a sedimentation coefficient close to 1 S. Experiments on FAM- rA_{18} binding to purified Hfq₆ show that the 3.8 S species corresponds to a 1:1 Hfq₆: FAM- rA_{18} complex (unpublished data). Thus, rA_{18} binds Hfq₆ displacing it from the complex constituting the 7.4 S peak. The small shoulder from 6 S to 8 S in the $g(s)$ distribution of Hfq-NA and FAM- rA_{18} monitored at 274 nm may reflect released nucleic acid.

Sedimentation equilibrium runs were next carried out with Hfq-NA at 12 K and 16 K rpm at concentrations of 1.75, 3.5, and 7 μ M moles Hfq hexamer (assuming absorbance at 274 nm is solely due to Hfq). Globally fitting the six data sets using Sedanal to a two species model yielded molecular weights of 68,977 <57,830, 78,263>, and 509,932 <327,529, 761,844>. Fig. 2B compares the non-linear least squares fits of the two-species model and one-species model to independent sedimentation equilibrium data. The model of two independent non-interacting components gave a much better fit than the single species model. The lower molecular weight species corresponds closely to the Hfq hexamer. The ~509 kD species correlates with the complex corresponding to the 7.4 S peak in the sedimentation velocity experiment and is much larger than a dimer of Hfq₆. Table S2 in supporting information summarizes sedimentation coefficients and experimentally derived molecular weights of the different Hfq species used in this study.

3.2. The nucleic acid contaminant is DNA

In order to determine the nature of the nucleic acid contributing to the 7.4 S peak, Hfq-NA was subjected to one of three treatments: (i) phenol-chloroform extraction to remove Hfq, (ii) digestion with DNase I prior to phenol-chloroform extraction, or (iii) digestion with RNase A prior to phenol-chloroform extraction. The aqueous phases from each of the above treatments were subjected to electrophoresis in a 1% agarose gel. The results indicate that DNA rather than RNA is the major nucleic acid component of Hfq-NA (Fig. 3). The mobility of the DNA corresponded to the mobility of duplex DNAs ranging from ~400 to 1000 bp. A simplified analysis of the UV spectrum of Hfq-NA, assuming this spectrum is a linear combination of purified Hfq and 50% GC duplex DNA indicate a very small amount of DNA contamination (~ 0.3%).

DNA isolated from Hfq-NA following the phenol-chloroform extraction and agarose gel separation was PCR amplified using the tagged random primers as described in Methods. The amplified duplex DNA was digested with *Bam*HI, cloned into pUC19 plasmid and sequenced. Table 1 lists sequence characteristics of 41 cloned DNAs. There were 24 different core sequences, 13 which were represented more than once, ranging in length from 60 to 567 bp. Most of the core sequences that were observed multiple times had slight differences at their ends (± 10 nts). This suggests they originated from different clones rather than a single plasmid. Four of the 24 fragments contained sequences from the expression plasmid. The others were from the *E. coli* genome. The average GC content of the *E. coli* fragments was 52.6%, close to the average for the *E. coli* genome (50.8%). The DNA sequences were distributed throughout most of the *E. coli* genetic map.

Thirteen of the 24 sequences in Table 1 had DNA helical axis curvature scores greater or equal to 10.0 using the BEND.IT algorithm [34,35]. Previous studies have shown that DNA sequences known to be curved in solution have scores greater than 9 [34,36]. The average calculated curvature score over the *E. coli* DNA genome is 7.7 [36]. The DNA sequence F11 which was found six times among the 41 clones had the highest curvature score, 15.5. The relatively high percentage of isolated segments with curvature scores 10 or higher

(27/41) is consistent with the previous indication that Hfq has a binding preference for curved DNA [14]. An experimental assessment of helical axis curvature was carried out on six DNA sequences in Table 1 by comparing their electrophoretic gel mobility against standard DNA lengths (Fig. S1). The ratio (L_R) of apparent length to actual length of four of the DNAs indicated significant helical axis curvature ($L_R > 1.15$). The other two DNAs examined had predicted curvature peaks near the ends of the duplexes which may explain their lower L_R values of 1.02 and 1.08.

3.3. Additional characteristics of the cloned DNA sequences

It was noted that 13 of the 20 *E. coli* DNA sequences in Table 1 (65%) were from genes identified as transport or membrane proteins. The annotated *E. coli* K12 strain has a total of 4793 genes with 538 or 11.2% identified as genes coding for membrane proteins (i.e. transport, symport, antiport, export, efflux, permease or channel proteins) [37]. If the DNA associated with Hfq represented random fragments of the *E. coli* genome it is highly unlikely 65% would be from membrane protein genes. Given the above number of total genes (4793) and membrane protein genes (538), application of the hypergeometric probability distribution indicates a probability of $\sim 10^{-8}$ for finding 13 membrane protein genes among 20 DNA segments by chance. Although this calculation assumes the cloned DNA segments are the size of genes, if one revisits the calculation assuming the DNA segments are 1/6 of an average gene length (as in this study), one obtains a similar probability.

A search was also made for conserved sequence elements among the *E. coli* DNA segments using the Gibbs Centroid Sampler algorithm [30]. This method found one to three copies of an 8 base consensus motif in 16 of the 20 segments. The sequence was (A/T)T(A/G)TGCCG with 78% to 100% identity to the consensus observed at each position for the 24 occurrences. The relationship of this motif and the other DNA characteristics on Hfq binding affinity are uncertain, but they suggest that Hfq may have a sequence and/or structure preference in its interaction with DNA.

3.4. Binding affinity of wt Hfq to DNA

The binding affinity of wt Hfq to the 352 bp H3-DNA and 241 bp F11-DNA was examined using the electrophoresis gel mobility shift assay (Fig. 4). The mobility of the shifted DNA bands decreased with increasing Hfq concentration suggesting multiple Hfq bind to each DNA. This interpretation is supported by the observation that addition of unlabeled DNA to preformed DNA-Hfq complexes increased the mobility of the observed complex with a concomitant increase in free DNA (data not shown). The concentration of Hfq₆ that shifted 50% of the initial amount of free F11-DNA and H3-DNA to lower mobility complexes was ~ 400 nM. This value is higher than values previously reported for shorter DNA fragments [14]. We note that the PCR generated H3-DNA shows two bands in the absence of Hfq. Separate experiments showed that single-strand specific exonuclease I removed the slower moving band, while heating and quick-cooling the H3-DNA greatly enhanced this band. This suggests that the slower moving band is a folded form of one of the strands of the H3-DNA duplex. The observation that this band shifts with the addition of Hfq implies that Hfq can bind to DNA with single-stranded character. Preliminary studies of Hfq binding to ten DNA single strands 60 to 80 nt long with various sequences indicate that Hfq has variable affinities for DNA strands with some showing little binding ($K_d > 2$ μ M) and one approaching an apparent $K_d \sim 200$ nM (unpublished data). We note that Mura *et al.* showed that Sm-like archeal proteins, which are related to Hfq, can bind supercoiled DNA and this interaction was inhibited by 20–30 nt single stranded DNAs [38].

In order to confirm that the DNA associated with Hfq during the isolation procedure is due to a specific interaction with Hfq a competition assay was carried out. PCR products were generated using the conditions described in Materials and methods from (1) DNA extracted from the Hfq-NA sample, and (2) *E. coli* K12 genomic DNA isolated from cells. The effect of competing these two unlabeled pools of DNA segments against the preformed ³²P-labeled F11–DNA–Hfq complex was examined by a gel-shift assay. Fig. S2 shows the results of this competition assay. 250 ng of PCR product from genomic DNA was needed to displace the F11–DNA–Hfq complex, while only 25 ng of product from the Hfq–NA DNA was required to displace the F11–DNA–Hfq complex. This result confirms that Hfq has a specific affinity for certain *E. coli* DNA sequences.

3.5. Hfq sites involved in binding DNA

The fact that rA₁₈ displaced Hfq from the Hfq–NA complexes in the sedimentation velocity experiment suggests that rA₁₈ and DNA interact with the same or overlapping Hfq binding sites. To help identify sites on Hfq that interact with DNA we examined the effect of altering Hfq residues on DNA binding. Nine mutant Hfq proteins each with a single residue changed to alanine and two truncated Hfq were expressed and purified as described in Materials and methods. Locations of the nine single residue changes are shown in Fig. 5. Hfq proteins designated Hfq-F39A, Hfq-F42A, Hfq-Q8A, Hfq-R16A, Hfq-R17A have single residue mutations on the proximal surface. Residues R16 and R17 also overlap onto the side or edge surface. The mutant Hfq designated Hfq-F11A and Hfq-R19A have mutations on the edge surface. Hfq-K31A and Hfq-Y25A have single residue mutations on the distal surface. Two mutant Hfq examined, Hfq-65 and Hfq-75, have all or part of the C-terminal domain deleted (Fig. S3). The structure of the C-terminal domain is unknown. The amino acid sequence (residues 66–102) suggests they are structurally disordered [39].

The relative affinity of the wt and mutant Hfq for two genomic DNA segments H3–DNA and F11–DNA was assessed using a gel shift assay (Fig. 6). 2 μM of each Hfq was added to 30 nM of each DNA. When wt Hfq was added to either DNA, approximately 65% of the free DNA band became a Hfq–DNA complex that migrated with low mobility. Hfq-F42A, Hfq-Q8A, Hfq-F39A and Hfq-R17A with alterations on the proximal surface also produced shifted bands although with varying characteristics. Hfq-F39A appears to bind both DNAs more weakly than wt Hfq since the shifted band has less intensity and is more diffuse. The mobility of the shifted bands for Hfq-42A and Hfq-R17A was faster than those for Hfq-39A, Hfq-Q8A and wt Hfq. Only the R16A mutation at the top of the proximal surface abolished Hfq binding to DNA.

Hfq with edge surface mutations F11A and R19A formed complexes with DNA. However both distal surface mutations, Y25A and K31A, eliminated Hfq binding to DNA. The latter result is consistent with the sedimentation velocity experiment in which rA₁₈ displaced DNA from Hfq. Both distal surface mutations were previously shown to affect Hfq binding to poly(rA) sequences [18,33,40].

A competition gel assay experiment in which DsrA or RprA is added to wt Hfq and F11–DNA provided additional evidence that Hfq's distal surface is involved in binding DNA. DsrA binds primarily to the proximal surface of Hfq [27,41], while RprA interacts with both proximal and distal surfaces [27]. When DsrA is added to a F11–DNA–Hfq complex, a super shift in the gel mobility shift assay is observed (Fig. S4A). This suggests Hfq can bind DsrA and DNA simultaneously. When RprA is added the F11–DNA–Hfq complex disappears and only free DNA is observed (Fig. S4B). This is consistent with RprA and F11–DNA sharing a common binding site on the distal surface.

Neither Hfq-65 nor Hfq-75 showed any significant binding to either DNA in this assay (Fig. 6). Previous studies show that Hfq-65 has similar, slightly reduced (2×) binding affinity to the RNAs DsrA and rA₂₇ compared to wt Hfq [42]. In the above assay both Hfq-65 and Hfq-75 shifted all DsrA and rA₁₈ to a complex (data not shown). Thus the C-terminus appears to be more involved in binding DNA than these RNAs. We note that all mutant Hfq's used in this study including Hfq-K31A and Hfq-Y25A can bind DsrA and RprA with apparent K_d 's varying from 20 to 150 nM [18,27,33], and can shift all of the sRNA to a complex (unpublished data).

4. Discussion

Hfq has been shown to be involved in various facets of RNA metabolism in the cell including sRNA ribo-regulation, and modulation of mRNA and sRNA half-life. Previous studies have demonstrated that Hfq also binds to DNA but with a weaker affinity than to RNA targets [14,17]. The current study set out to characterize what was thought to be RNA contaminants and revealed the contaminant to be predominantly genomic DNA from the host strain. The results support previous work indicating Hfq binds DNA [14,17] and provides new information on the nature of Hfq–DNA interaction.

The sedimentation velocity and sedimentation equilibrium data together with the DNase digestion results indicate that Hfq–DNA complexes are responsible for the 7.4 S sedimentation species. Addition of Hfq to duplex DNAs caused a steady decrease in gel mobility of the Hfq–DNA complexes, consistent with multiple Hfq hexamers binding these DNA probes. The average molecular weight of the Hfq–DNA complexes of Hfq–NA estimated from sedimentation equilibrium analysis, 509 kD, also suggests several Hfq₆ per complex for DNAs~500 bp in length.

The ability of rA₁₈ to disrupt the Hfq–DNA complex in the sedimentation velocity experiment suggests that DNA binds to the distal surface of Hfq. The observation that two amino acid residues on the distal side of Hfq are important for DNA binding, and the influence of DsrA and RprA on Hfq–DNA complexes supports this hypothesis. The distal surface of *E. coli* Hfq₆ is known to be positively charged [3] and this property could help drive its association with DNA. We note however that isolation of Hfq–NA involved extensive washing with 1 M NaCl buffer, and the sedimentation experiments were conducted in 0.2 M NaCl or 0.5 M NaCl solutions with similar outcomes. The stability of Hfq–DNA complexes under these conditions implies that non-specific electrostatic interactions do not dominate Hfq–DNA binding.

Most mutant Hfq with residue changes on the proximal and edge surfaces exhibited Hfq–DNA complexes with a gel mobility that was the same as the wild type Hfq–DNA complex (Fig. 6). The mobility of the Hfq–DNA complexes involving Hfq-R17A and Hfq-F42A differed. Since complexes formed by Hfq-R17A and Hfq-F42A with RprA and DsrA have gel mobilities identical to wt Hfq ([27] and unpublished data), the faster mobility of these mutant Hfq–DNA complexes appears to reflect aspects of Hfq–DNA interactions rather than a general defect in multiple Hfq binding to nucleic acids. Interestingly, Hfq-R16A, a mutation at the interface of the proximal and edge surface, did not bind DNA. This mutation reduces Hfq binding to domain II of DsrA by 7 fold, but it has a minimal effect on Hfq binding to RprA [18,27].

Both truncated forms of Hfq, Hfq-65 and Hfq-75, did not bind DNA in the gel assay. The C-terminal domain of *E. coli* Hfq has been shown to stabilize its hexamer form [43] and truncating them could influence DNA-or RNA-binding indirectly by reducing the amount of Hfq in hexamer form. While this cannot be ruled out, the same gel shift assay carried out

with these truncated Hfq and rA₁₈ or DsrA showed both RNAs shifting completely to a complex (unpublished data). Residues of the C-terminus beyond position 75 are involved directly or indirectly in binding DNA.

The sequence analysis of the DNA fragments associated with Hfq revealed several interesting characteristics. 27 of the 41 cloned DNA segments had a helical axis curvature score of 10 or higher compared to an average of 7.7 for the *E. coli* genome. The gel mobility of several of the DNA fragments verified they had significant helical axis curvature. While helical axis curvature appears to be correlated with Hfq affinity to DNA, it is unlikely to be the sole determinant. Prediction of helical axis curvature in the *E. coli* DNA genome using the BEND.IT algorithm indicates ~20% of DNA segments have curvature scores of 10 or higher and 4.7% have scores of 15 or higher [36,44]. It is nonetheless of interest to note that an analysis of the 5% most curved DNA regions of the *E. coli* genome shows they are preferentially located 100 to 200 bases upstream of the nearest start codon [44]. While the results of this work do not address the functional significance of Hfq–DNA interaction, one may speculate that for some genes Hfq binding to DNA regions near promoters could be coupled to Hfq binding to and regulation of the corresponding transcribed mRNA. A recent work implied that Hfq affects the transcription of genes as well as influences post-transcriptional events [45].

A second perhaps more significant feature of the DNA associated with Hfq was the observation that a majority of the *E. coli* DNA segments (13 of 20) was from genes coding for membrane proteins. The estimated probability of this occurring by chance is extremely low ($\sim 10^{-8}$). A recent study showed Hfq is located close to the inner membrane of *E. coli* as well as in internal regions of the cell when expressed at levels corresponding to the stationary phase of growth [16]. When overexpressed, Hfq is also located at the outer membrane. It will be of interest to determine if the later characteristic is related to Hfq overexpression or if it occurs during physiological levels of Hfq expression [39].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge Xueguang Sun and Charles Terry in the purification of mutant and wt Hfq proteins used in this study and King Jordan and Jianrong Wang for helpful discussions on sequence analysis. We gratefully acknowledge funding from the NASA Astrobiology Institute.

Abbreviations

nt	nucleotides
sRNA	small RNA
bp	base pairs
PAGE	polyacryl-amide gel electrophoresis
MWCO	molecular weight cut-off
FAM	6-carboxyfluorescein

References

1. Valentin-Hansen P, Eriksen M, Udesen C. The bacterial Sm-like protein Hfq: a key player in RNA transactions. *Mol Microbiol.* 2004; 51:1525–1533. [PubMed: 15009882]

2. Majdalani N, Vanderpool CK, Gottesman S. Bacterial small RNA regulators. *Crit Rev Biochem Mol Biol.* 2005; 40:93–113. [PubMed: 15814430]
3. Brennan RG, Link TM. Hfq structure, function and ligand binding. *Curr Opin Microbiol.* 2007; 10:125–133. [PubMed: 17395525]
4. Jousset A, Metzinger L, Felden B. On the facultative requirement of the bacterial RNA chaperone, Hfq. *Trends Microbiol.* 2009; 17:399–405. [PubMed: 19733080]
5. Papenfort K, Vogel J. Multiple target regulation by small noncoding RNAs rewires gene expression at the post-transcriptional level. *Res Microbiol.* 2009; 160:278–287. [PubMed: 19366629]
6. Waters LS, Storz G. Regulatory RNAs in bacteria. *Cell.* 2009; 136:615–628. [PubMed: 19239884]
7. Tsui HC, Leung HC, Winkler ME. Characterization of broadly pleiotropic phenotypes caused by an hfq insertion mutation in *Escherichia coli* K-12. *Mol Microbiol.* 1994; 13:35–49. [PubMed: 7984093]
8. Ding Y, Davis BM, Waldor MK. Hfq is essential for *Vibrio cholerae* virulence and downregulates sigma expression. *Mol Microbiol.* 2004; 53:345–354. [PubMed: 15225327]
9. 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. Involvement of the post-transcriptional regulator Hfq in *Yersinia pestis* virulence. *PLoS ONE.* 2009; 4:e6213. [PubMed: 19593436]
10. Meibom KL, Forslund AL, Kuoppa K, Alkhuder K, Dubail I, Dupuis M, Forsberg A, Charbit A. Hfq, a novel pleiotropic regulator of virulence-associated genes in *Francisella tularensis*. *Infect Immun.* 2009; 77:1866–1880. [PubMed: 19223477]
11. Sonnleitner E, Hagens S, Rosenau F, Wilhelm S, Habel A, Jager KE, Blasi U. Reduced virulence of a hfq mutant of *Pseudomonas aeruginosa* O1. *Microb Pathog.* 2003; 35:217–228. [PubMed: 14521880]
12. Robertson GT, Roop RM Jr. 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.* 1999; 34:690–700. [PubMed: 10564509]
13. Kajitani M, Kato A, Wada A, Inokuchi Y, Ishihama A. Regulation of the *Escherichia coli* hfq gene encoding the host factor for phage Q beta. *J Bacteriol.* 1994; 176:531–534. [PubMed: 8288550]
14. Azam TA, Ishihama A. Twelve species of the nucleoid-associated protein from *Escherichia coli*, sequence recognition specificity and DNA binding affinity. *J Biol Chem.* 1999; 274:33105–33113. [PubMed: 10551881]
15. Azam TA, Hiraga S, Ishihama A. Two types of localization of the DNA-binding proteins within the *Escherichia coli* nucleoid. *Genes Cells.* 2000; 5:613–626. [PubMed: 10947847]
16. Diestra E, Cayrol B, Arluison V, Risco C. Cellular electron microscopy imaging reveals the localization of the Hfq protein close to the bacterial membrane. *PLoS ONE.* 2009; 4:e8301. [PubMed: 20011543]
17. Takada A, Wachi M, Kaidow A, Takamura M, Nagai K. DNA binding properties of the hfq gene product of *Escherichia coli*. *Biochem Biophys Res Commun.* 1997; 236:576–579. [PubMed: 9245691]
18. Sun X, Wartell RM. *Escherichia coli* Hfq binds A18 and DsrA domain II with similar 2:1 Hfq6/RNA stoichiometry using different surface sites. *Biochemistry.* 2006; 45:4875–4887. [PubMed: 16605255]
19. Hemsley A, Arnheim N, Toney MD, Cortopassi G, Galas DJ. A simple method for site-directed mutagenesis using the polymerase chain reaction. *Nucleic Acids Res.* 1989; 17:6545–6551. [PubMed: 2674899]
20. Liu S, Stafford WF III. An optical thermometer for direct measurement of cell temperature in the Beckman instruments XL-A analytical ultracentrifuge. *Anal Biochem.* 1995; 224:199–202. [PubMed: 7710072]
21. Schuck P, Perugini MA, Gonzales NR, Howlett GJ, Schubert D. Size-distribution analysis of proteins by analytical ultracentrifugation: strategies and application to model systems. *Biophys J.* 2002; 82:1096–1111. [PubMed: 11806949]
22. Philo JS. Improved methods for fitting sedimentation coefficient distributions derived by time-derivative techniques. *Anal Biochem.* 2006; 354:238–246. [PubMed: 16730633]

23. Stafford WF, Sherwood PJ. Analysis of heterologous interacting systems by sedimentation velocity: curve fitting algorithms for estimation of sedimentation coefficients, equilibrium and kinetic constants. *Biophys Chem.* 2004; 108:231–243. [PubMed: 15043932]
24. Laue, TM.; Shah, BD.; Ridgeway, TM.; Pelletier, SL. Analytical ultracentrifugation in biochemistry and polymer sciences. Harding, SE.; Rowe, AJ.; Horton, JC., editors. Royal Society of Chemistry; Cambridge, U.K: 1992.
25. Grothues D, Cantor CR, Smith CL. PCR amplification of megabase DNA with tagged random primers (T-PCR). *Nucleic Acids Res.* 1993; 21:1321–1322. [PubMed: 8464718]
26. Diekmann S. Analyzing DNA curvature in polyacrylamide gels. *Methods Enzymol.* 1992; 212:30–46. [PubMed: 1518451]
27. Updegrave T, Wilf N, Sun X, Wartell RM. Effect of Hfq on RprA-rpoS mRNA pairing: Hfq-RNA binding and the influence of the 5' rpoS mRNA leader region. *Biochemistry.* 2008; 47:11184–11195. [PubMed: 18826256]
28. Brukner I, Sanchez R, Suck D, Pongor S. Sequence-dependent bending propensity of DNA as revealed by DNase I: parameters for trinucleotides. *EMBO J.* 1995; 14:1812–1818. [PubMed: 7737131]
29. Gabrielian A, Pongor S. Correlation of intrinsic DNA curvature with DNA property periodicity. *FEBS Lett.* 1996; 393:65–68. [PubMed: 8804425]
30. Thompson WA, Newberg LA, Conlan S, McCue LA, Lawrence CE. The Gibbs centroid sampler. *Nucleic Acids Res.* 2007; 35:W232–W237. [PubMed: 17483517]
31. Carmichael GG, Weber K, Niveleau A, Wahba AJ. The host factor required for RNA phage Qbeta RNA replication in vitro. Intracellular location, quantitation, and purification by polyadenylate-cellulose chromatography. *J Biol Chem.* 1975; 250:3607–3612. [PubMed: 805130]
32. Arluison V, Derreumaux P, Allemand F, Folichon M, Hajnsdorf E, Regnier P. Structural Modelling of the Sm-like Protein Hfq from *Escherichia coli*. *J Mol Biol.* 2002; 320:705–712. [PubMed: 12095248]
33. Mikulecky PJ, Kaw MK, Brescia CC, Takach JJ, Sledjeski D, Feig AL. *Escherichia coli* Hfq has distinct interaction surfaces for DsrA, rpoS and poly(A) RNAs. *Nat Struct Mol Biol.* 2004; 11:1206–1214. [PubMed: 15531892]
34. Munteanu MG, Vlahovicek K, Parthasarathy S, Simon I, Pongor S. Rod models of DNA: sequence-dependent anisotropic elastic modelling of local bending phenomena. *Trends Biochem Sci.* 1998; 23:341–347. [PubMed: 9787640]
35. Vlahovicek K, Kajan L, Pongor S. DNA analysis servers: plot.it, bend.it, model.it and IS. *Nucleic Acids Res.* 2003; 31:3686–3687. [PubMed: 12824394]
36. Gabrielian A, Vlahovicek K, Pongor S. Distribution of sequence-dependent curvature in genomic DNA sequences. *FEBS Lett.* 1997; 406:69–74. [PubMed: 9109388]
37. Durfee T, Nelson R, Baldwin S, Plunkett G III, Burland V, Mau B, Petrosino JF, Qin X, Muzny DM, Ayele M, Gibbs RA, Csorgo B, Posfai G, Weinstock GM, Blattner FR. The complete genome sequence of *Escherichia coli* DH10B: insights into the biology of a laboratory workhorse. *J Bacteriol.* 2008; 190:2597–2606. [PubMed: 18245285]
38. Mura C, Kozhukhovskiy A, Gingery M, Phillips M, Eisenberg D. The oligomerization and ligand-binding properties of Sm-like archaeal proteins (SmAPs). *Protein Sci.* 2003; 12:832–847. [PubMed: 12649441]
39. Sauter C, Basquin J, Suck D. Sm-like proteins in Eubacteria: the crystal structure of the Hfq protein from *Escherichia coli*. *Nucleic Acids Res.* 2003; 31:4091–4098. [PubMed: 12853626]
40. Arluison V, Mutyam SK, Mura C, Marco S, Sukhodolets MV. Sm-like protein Hfq: location of the ATP-binding site and the effect of ATP on Hfq–RNA complexes. *Protein Sci.* 2007; 16:1830–1841. [PubMed: 17660259]
41. Brescia CC, Mikulecky PJ, Feig AL, Sledjeski DD. Identification of the Hfq-binding site on DsrA RNA: Hfq binds without altering DsrA secondary structure. *Rna.* 2003; 9:33–43. [PubMed: 12554874]
42. Vecerek B, Rajkowitsch L, Sonnleitner E, Schroeder R, Blasi U. The C-terminal domain of *Escherichia coli* Hfq is required for regulation. *Nucleic Acids Res.* 2008; 36:133–143. [PubMed: 18000007]

43. Arluison V, Folichon M, Marco S, Derreumaux P, Pellegrini O, Seguin J, Hajnsdorf E, Regnier P. The C-terminal domain of *Escherichia coli* Hfq increases the stability of the hexamer. *Eur J Biochem.* 2004; 271:1258–1265. [PubMed: 15030475]
44. Bolshoy A, Nevo E. Ecologic genomics of DNA: upstream bending in prokaryotic promoters. *Genome Res.* 2000; 10:1185–1193. [PubMed: 10958636]
45. Le Derout J, Boni IV, Regnier P, Hajnsdorf E. Hfq affects mRNA levels independently of degradation. *BMC Mol Biol.* 11:17. [PubMed: 20167073]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.bbagr.2010.06.007.

Fig. 1.

A. UV spectra of Hfq-NA, prior to nuclease treatment (—), and after nuclease treatment (.....). B. Normalized g(s) distribution of sedimentation velocity runs monitored at 274 nm of purified Hfq (black line), and Hfq-NA samples (red/black line).

Fig. 2.

A. Effect of adding 3 μ M FAM-A18 on sedimentation velocity distribution of 3 μ M Hfq-NA; distribution of Hfq-NA alone monitored at 274 nm (red/black line), distribution of Hfq-NA and FAM-A18 monitored at 274 nm (black dotted line) and monitored at 495 nm (green line). B. Sedimentation equilibrium profile of Hfq-NA sample (oooo) and non-linear least squares fit of one component (- - -) and two component models (—). The one component fit yielded a M.W. of 79842. The two component model yielded M.W. of 66,313 and 531,032 for the two species. The latter values are in very good agreement with a global fit to data obtained with another Hfq-NA sample (see text).

Fig. 3.
1% agarose gel of aqueous phase of phenol extracted Hfq-NA samples first treated with RNase A (lane 1), DNase I (lane 2), or untreated (lane 3). DNA markers are in lane 4.

Fig. 4. Hfq binding to ^{32}P -labeled DNA fragments (4 nM) assessed by the gel mobility shift assay. Hfq₆ concentrations added varied from 0 to 2 μM . A. Titration of the 241 bp F11-DNA. B. Titration of the 352 bp H3-DNA.

Fig. 5. Space filling model of the toroidal part of *E. coli* Hfq₆ showing the locations of the nine single residue mutations examined from the proximal, distal and edge surfaces.

Fig. 6. Gel shift assay of the relative affinity of wild type and eleven mutant Hfq₆ for; A. H3-DNA, and B. F11-DNA. 2 μ M of each Hfq was added to 30 nM DNA in each lane except for the DNA only control lanes.

Table 1

Sequence characteristics of DNA segments amplified from DNA associated with Hfq.

DNA	Locus	Gene/functional description	Length (bp)	Copies	Curvature scores		% GC
					Dnase	Consensus	
G1	2434398..2433909	<i>nrkA</i> /ribonucleoside diphosphate reductase 1, α subunit	491	2	8.5	9	52
H1	2787761..2788044	<i>yfiB</i> /conserved hypothetical protein	283	1	11	10	53
H3	335274..335626	* <i>sbmA</i> /ABC transporter trans-membrane	352	2	11.5	11	53
H5	4550393..4550611	* <i>yjiR</i> /predicted sugar transporter subunit, ATP-binding	218	2	9	7	58
F1	3185823..3186066	<i>gshB</i> /glutathione synthetase	243	2	7.5	8	50
F2	1132955..1133187	* <i>putP</i> /sodium/proline symporter	232	3	12	10	51
F5	4382281..4382555	* <i>actP</i> /acetate symporter (acetate transporter)	274	2	10	9	52
F8	3986385..3986781	* <i>tnaB</i> /tryptophan transporter of low affinity	396	2	10	9.5	49
B7	3830968..3831226	<i>xyiR</i> /DNA-binding transcriptional activator; AraC family	258	2	13	9.5	52
B8	2325399..2325946	* <i>mgIC</i> /methyl-galactoside transporter subunit	547	2	13	11	51
B9	506621..507026	* <i>emrE</i> /DLP12 prophage; multidrug resistance protein	405	1	15	11	43
D12	4361318..4361637	<i>qor</i> /quinone oxidoreductase, NADPH-dependent	319	1	10	9	54
F10	3938383..3938530	* <i>yicO</i> /predicted xanthine/uracil permease	147	1	5.5	4.5	54
F11	3706138..3706379	* <i>yhhS</i> /periplasmic protein; putative transporter	241	6	15.5	12	52
F15	4005299..4005492	* <i>pstA</i> /phosphate transporter subunit	193	2	6.5	7	55
F16	1444080..1444470	* <i>sapB</i> /antimicrobial peptide transporter subunit	390	1	11	8	53
H4	4673798..4673858	* <i>yjiK</i> /predicted transporter subunits of ABC superfamily	60	2	7	6	52
D11	2647738..2648025	<i>eutB</i> /ethanolamine ammonia-lyase	287	1	12	13	58
D11'	2657140..2657417	* <i>eutH</i> /predicted inner membrane protein	277	1	9	8	53
C4	2980403..2980970	<i>cysJ</i> /sulfite reductase, alpha subunit, flavoprotein	567	1	8.5	8	56
H1'	Expression plasmid	pTYB11 sequence	200	3	13	12	54
F1'	Expression plasmid	pTYB11 sequence	183	1	9.5	7.5	49
D12'	Expression plasmid	pTYB11 sequence	230	1	9.5	7.5	52
F11'	Expression plasmid	pTYB11 sequence	199	1	9.5	7.5	49

First column lists DNA designation. DNA segments from the same plasmid share the same letter, e.g. D11, D11'. Locus refers to the sequence of the genome of *E. coli* str. K12 substr. D10B given at NCBI. Some DNA sequences were found multiple times (copies). Curvature scores refer to the peak scores calculated with the two parameter sets available at the BEND. IT server [34,35]. "Dnase" refers to scores obtained using the dinucleotide bendability parameters determined from DNase I digest data [28], while "Consensus" refers to scores obtained using consensus dinucleotide bendability parameters (Dnase I digest and nucleosome positioning data [29]). DNAs coding for transport or membrane proteins are noted with a *.