

## *Legend*

*Figure1 ; Predicted structures of the RNAs used in the gel retardation experiments.* The RNA sequences were folded with *mfold* (<http://www.bioinfo.rpi.edu/applications/mfold>). The secondary structure of the 3' *rpsO*-A18 mRNA fragment was experimentally determined (1). Enzymatic probing of a slightly longer *hfq* mRNA fragment revealed that most of the secondary structures predicted by *mfold* are not stable in solution (2).

Table 1 : Primers used to perform mutagenesis

Resulting plasmid	Template	Primer sequence
pTE607D40A	pTE607	For:GCTGCAAGGGCAAATCGAGTCTTTTGCTCA GTTTCGTGATCCTGTTGAAAAACAC Rev:GTGTTTTTCAACAGGATCACGAACTGAGCA AAAGACTCGATTTGCCCTTGCAGC
pTE607F42A	pTE607	For:GGCAAATCGAGTCTTTTGATCAGGCTGTGAT CCTGTTGAAAAACACGGTC Rev:GACCGTGTTTTTCAACAGGATCACAGCCTG ATCAAAAGACTCGATTTGCC
pTE607K56A	pTE607	For:CACGGTCAGCCAGATGGTTTACGCTCACGC GATTTCTACTGTTGTCCCGTC Rev:GACGGGACAACAGTAGAAATCGCGTGAGC GTAAACCATCTGGCTGACCGTG
pTE607D40A-F42A	pTE607-F42A	For:GGCAAATCGAGTCTTTTGCTCAGGCTGTGAT CCTGTTGAAAAACACGGTC Rev;GACCGTGTTTTTCAACAGGATCACAGCCTG AGCAAAGACTCGATTTGCC
pTE607V43C	pTE607	For:GGCAAATCGAGTCTTTTGATCAGTTCTGTAT CCTGTTGAAAAACACGGT Rev;GACCGTGTTTTTCAACAGGATACAGAACTG ATCAAAAGACTCGATTTGCC
pTE607V43R	pTE607	For:GGCAAATCGAGTCTTTTGATCAGTTCCGCAT CCTGTTGAAAAACACGGTC Rev;GACCGTGTTTTTCAACAGGATGCGGAACTG ATCAAAAGACTCGATTTGCC
pTX381V43C	pTX381	For:GGCAAATCGAGTCTTTTGATCAGTTCTGTAT CCTGTTGAAAAACACGGT Rev;GACCGTGTTTTTCAACAGGATACAGAACTG ATCAAAAGACTCGATTTGCC
pTX381V43R	pTX381	For:GGCAAATCGAGTCTTTTGATCAGTTCCGCAT CCTGTTGAAAAACACGGTC Rev;GACCGTGTTTTTCAACAGGATGCGGAACTG ATCAAAAGACTCGATTTGCC

Table 2 ; *E. coli* strains used in this work.

Strain	Relevant Genotype	Source or Reference
TX2808	JC7623 <i>hfq1</i> :: $\Omega$ (kan <sup>R</sup> )	(3)
HAT10	<i>hfq10</i> ::cat (cm <sup>R</sup> )	(4)
RO91	<i>rpoS742</i> :: <i>lacZ</i>	(5)
MCM11	RO91 <i>hfq10</i> ::cat (cm <sup>R</sup> )	M. Springer
BL21 $\lambda$ DE3 <i>hfq1</i>	BL21 $\lambda$ DE3 <i>hfq1</i> :: $\Omega$ □□□□	This study
N3433	<i>HfrH lacZ43 relA1 spoT1 thi-1</i>	D. Apirion
CAG12073	<i>cycA30</i> :: <i>Tn10</i> (tet <sup>R</sup> )	(6)
MA261 <i>oppA</i> ::Km		(7)
IBPC937	TX2808 <i>hfqV43R</i> (kan <sup>S</sup> )	This study
IBPC946	TX2808 <i>hfq</i> $\Delta$ 22-294 (kan <sup>S</sup> )	This study
IBPC929	N3433 <i>hfq1</i> :: $\Omega$ (kan <sup>R</sup> , <i>BclI</i> )	This study
IBPC941	N3433 <i>hfqV43R cycA30</i> :: <i>Tn10</i> (tet <sup>R</sup> )	This study
IBPC953	N3433 <i>hfq</i> $\Delta$ 22-294 <i>cycA30</i> :: <i>Tn10</i> (tet <sup>R</sup> )	This study
IBPC959	IBPC953 <i>oppA</i> ::km (kan <sup>R</sup> )	This study
ENSO	former name HfrG6 $\Delta$ 12	(8)
IBhfq95	ENSO <i>hfq</i> :: <i>lacZ</i>	This study
IBhfq95- <i>hfqV43R</i>	ENSO <i>hfq</i> :: <i>lacZ hfqV43R</i>	This study

Table 3; Statistics of 1HK9 (wt, V43C, V43R)

Simulation	$\alpha$ -helix $\pm \sigma_{\alpha}\%$	$\beta$ -strand $\pm \sigma_{\beta}\%$	H	B1	B2	B3	B4	B5
Initial structure	16.2	46.4	100	100	100	100	100	100
wt	16.2 $\pm$ 0.4	45.5 $\pm$ 1.4	92.1	95.9	89.4	92.8	89.8	93.1
V43C	15.8 $\pm$ 0.3	46.3 $\pm$ 1.2	90.2	96.2	91.2	95.7	90.9	94.3
V43R	15.1 $\pm$ 0.4	41.7 $\pm$ 1.6	90.5	96.0	84.4	85.9	74.6	92.1

The mean and standard deviation ( $\sigma$ ) of  $\alpha$ -helix and  $\beta$ -strand are given for the minimized (initial) structure and for the time-averaged wt, V43C and V43R structures. The mean percentage of helix (H) and  $\beta$ -strands (B1 to B5) are shown to the right.

### References

1. Folichon, M., Arluison, V., Pellegrini, O., Huntzinger, E., Regnier, P. and Hajsndorf, E. (2003) The poly(A) binding protein Hfq protects RNA from RNase E and exoribonucleolytic degradation. *Nucleic Acids Res*, **31**, 7302-7310.
2. Vecerek, B., Moll, I. and Blasi, U. (2005) Translational autocontrol of the *Escherichia coli* hfq RNA chaperone gene. *Rna*, **11**, 976-984.
3. Tsui, H.-C., T., Leung, H.-C., E. and Winkler, M.E. (1994) Characterization of broadly pleiotropic phenotypes caused by an hfq insertion mutation in *Escherichia coli* K-12. *Mol. Microbiol.*, **13**, 35-49.
4. Wachi, M., Takada, A. and Nagai, K. (1999) Overproduction of the outer-membrane proteins FepA and FhuE responsible for iron transport in *Escherichia coli* hfq::cat mutant. *Biochem. Biophys. Res. Com.*, **264**, 525-529.
5. Lange, R. and Hengge-Aronis, R. (1994) The cellular concentration of the  $\sigma^S$  subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation and protein stability. *Genes & Dev.*, **8**, 1600-1612.
6. Singer, M., Baker, T.A., Schnitzler, G., Deischel, S.M., Goel, M., Dove, W., Jaacks, K.J., Grossman, A.D., Erickson, J.W. and Gross, C.A. (1989) A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. *Microbiol Rev*, **53**, 1-24.
7. Igarashi, K., Saisho, T., Yuguchi, M. and Kashiwagi, K. (1997) Molecular mechanism of polyamine stimulation of the synthesis of oligopeptide-binding protein. *J Biol Chem*, **272**, 4058-4064.
8. Dreyfus, M. (1988) What constitutes the signal for the initiation of protein synthesis on *Escherichia coli* mRNAs? *J Mol Biol*, **204**, 79-94.