doi:10.1016/j.jmb.2010.09.038 J. Mol. Biol. (2010) 404, 173–182

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

COMMUNICATION

C-Terminally Truncated Derivatives of *Escherichia coli* Hfq Are Proficient in Riboregulation

Anders Steno Olsen[1], Jakob Møller-Jensen[1], Richard G. Brennan[2] and Poul Valentin-Hansen[1]*

[1]*Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark*
[2]*Department of Biochemistry and Molecular Biology, University of Texas M. D. Anderson Cancer Center, Houston, TX, USA*

Received 18 June 2010; received in revised form 16 September 2010; accepted 18 September 2010
Available online 1 October 2010

Edited by D. E. Draper

Keywords:
Hfq;
role of C-terminus;
rpoS expression;
sRNA regulation;
posttranscriptional control

The prokaryotic Sm-like protein Hfq plays an essential role in the stability and function of trans-encoded small regulatory RNAs in enterobacteria that function in posttranscriptional control by base-pairing with cognate target mRNAs. Hfq associates with both regulatory RNA and target RNA, and its interaction promotes annealing. So far, mutational and structural studies have established that *Escherichia coli* Hfq contains two separate RNA binding sites that are part of the conserved N-terminal portion of the protein. Moreover, it has been suggested that the nonconserved C-terminal extension of *E. coli* Hfq might constitute a third RNA interaction surface with specificity for mRNA. However, the role of the C-terminus has not been fully resolved but is clearly important for a complete understanding of Hfq function in posttranscriptional regulation and RNA decay. Here we examined the ability of *E. coli* Hfq derivatives, consisting of the conserved core and short C-terminal extensions, to support the regulation of *rpoS* expression and riboregulation by various well-characterized small regulatory RNAs. Our data show that, in all cases tested, the truncated proteins are fully capable of promoting posttranscriptional control, indicating that the C-terminal tail of *E. coli* Hfq plays a small role or no role in riboregulation.

The genomes of many prokaryotes encode a single protein or, at most, a few proteins that belong to the Sm/Lsm superfamily of structurally and functionally related RNA-binding proteins of ancient origin.[1] Structural and biochemical studies on several of these proteins have provided general insights into their oligomerization behavior and RNA binding properties.[2,3] Members of the Sm/Lsm family, including bacterial Hfq, possess a common bipartite motif of approximately 70 amino acid residues consisting of two relatively conserved regions, termed Sm1 and Sm2 motifs (Fig. 1a). These motifs are separated by a spacer, which is not conserved in its sequence or length.[9,10] Crystal structures of Sm proteins established that the two signature sequences constitute an autonomously folded domain composed of a bent five-stranded antiparallel β-sheet (which is responsible for RNA binding and oligomerization into doughnut-shaped

*Corresponding author. E-mail address: valentin@bmb.sdu.dk.

Abbreviation used: sRNA, small regulatory RNA.

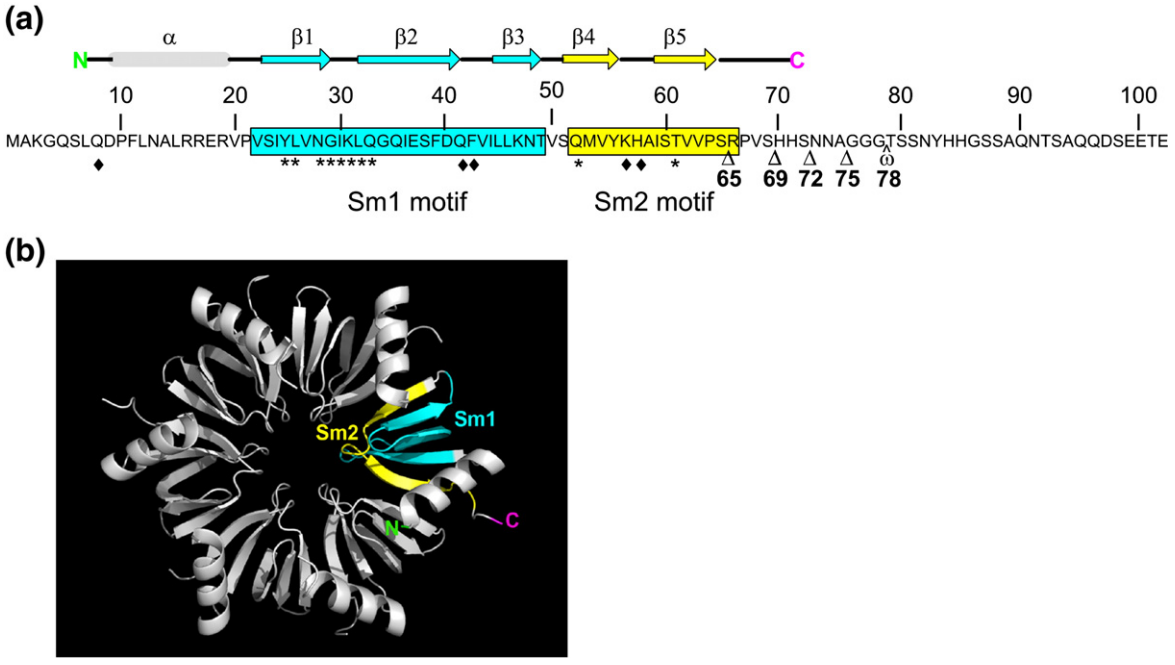

Fig. 1. *E. coli* Hfq. (a) Sequence of *E. coli* Hfq protein. The location of Sm1 and Sm2 sequence motifs is indicated by boxes, and the open arrows correspond to the C-terminus of the short Hfq forms. The *hfq-2* allele described by Tsui *et al*. bears an omega cassette after codon 78, which is denoted by the ω symbol.[4] The Hfq core (residues 7–66) is common to bacterial proteins.[5] Residues forming the A/U binding pockets (determinants of the proximal binding site) and residues forming the (ARN)/(ARNN′) pockets (determinants of the distal binding site) in the crystal structures of Hfq–RNA complexes are labelled with filled diamonds and asterisks, respectively.[6,7] (b) Ribbon diagram of *E. coli* Hfq. The view on the "proximal" face of the hexamer. The secondary structures of each subunit are depicted as coils for α-helices and as arrows for β-strands. The conserved Sm1 and Sm2 motifs of one subunit are labelled and shown in cyan and yellow, respectively. The N-terminus and the C-terminus of the subunit are labelled and shown in green and magenta, respectively. The figure was generated with PyMOL[8] using coordinates with Protein Data Bank accession code 3GIB.

structures) and an N-terminal α-helix (Fig. 1b).[11–15] This fold is remarkably conserved among eukaryotic, archaeal, and bacterial proteins, despite considerable variations in primary amino acid sequence.[6,16]

Hfq is present in many Gram-negative and Gram-positive bacteria, as well as in the archaeon *Methanococcus jannaschii*,[16] and varies in length from approximately 70 to 100 amino acids, although a few Hfq proteins, including those from *Moraxella catarrhalis* and *Acinetobacter baylyi*, are significantly longer.[17–19] At present, the best-studied bacterial Sm-like protein is Hfq from *Escherichia coli*, and the best-characterized function of this protein is its role in mediating the posttranscriptional effects of small regulatory RNAs (sRNAs).[5,20–23] Unlike heptameric Sm proteins, Hfq protomers assemble to form highly stable hexamers, which have a binding preference for A/U-rich tracts on target RNAs[6,24–26]—an interaction that stabilizes many sRNAs *in vivo*. Moreover, the protein promotes the formation of binary sRNA–target mRNA complexes *in vitro* by increasing the on-rate of duplex formation.[27–33]

The precise mechanism(s) by which Hfq functions is still not fully understood. However, biochemical, mutational, and structural studies have established that Hfq contains at least two separate RNA binding sites.[6,7,34,35] One is situated on the "proximal side" and consists of six essentially identical binding pockets that can accommodate U-nucleotides or A-nucleotides. One mechanism of Hfq binding to U-rich oligoribonucleotides was revealed in the structure of *Staphylococcus aureus* Hfq bound to the heptamer 5′-AUUUUUG. In this complex, the RNA is bound in a circular unwound manner around the pore of the Hfq hexamer, within a basic patch.[6] A second independent RNA binding site is present on the "distal" side and is responsible for the high-affinity binding of poly(A) tails by Hfq. In the structure of a C-terminal truncation mutant of *E. coli* Hfq, Hfq_{69} (lacking residues 70–102), which is bound to the oligoribonucleotide A_{15}, the poly(A) tract also binds in a circular manner, utilizing six