Identifying RNA binding sites on the *E. coli* Hfq protein

X. Sun, and R. M. Wartell*
School of Biology, Georgia Institute of Technology, Atlanta Georgia 30332

Hfq is a RNA-binding protein originally discovered as a host factor required for Qβ RNA virus replication in *E. coli*. The *hfq* gene is highly conserved among bacterial phyla and the protein has been shown to play an important role in regulating translation of a number of mRNAs. In *E. coli* it binds to small regulatory RNAs (e.g, DsrA, OxyS, RprA) and is required for the interaction of regulatory RNAs with their targeted mRNAs. Studies indicate that Hfq functions by enhancing bimolecular RNA-RNA pairing. Crystal structures of *S. aureus* and *E. coli* Hfq reveal that the protein forms a donut-like hexamer, and a structure of *S. aureus* Hfq with a U-rich RNA heptamer shows a RNA binding site that follows a circular path on one surface of Hfq. In this structure the highly conserved Tyr at position 42 of each Hfq subunit stack alternately between six of the bases that extend from the circular path of the RNA backbone. Comparative sequence analysis and structure modeling suggest the location of a second RNA-binding site. The path of this site involves a concentric ring of residues at a larger radius. In nearly all bacterial Hfq sequences (~160) Phe or Tyr occurs at position 39 forming a concentric ring of aromatic residues at the larger radius on the same Hfq surface. A small aliphatic group co-varies at position 12 adjacent to position 39 in the 3-D Hfq structure. In every instance (~ 6) where Phe or Tyr does not occur at position 39, Tyr replaces the small aliphatic group at position 12. To test the role of these residues, wild type *E. coli* Hfq and Hfq mutated at positions 12, 39, and 42 and other positions were over-expressed and their binding to rA_{18} and several other RNA oligomers were examined by protein fluorescence quenching, gel retardation and fluorescence anisotropy. Results show that the F42A mutation (the inner ring) does not influence the binding of rA_{18} to Hfq, whereas the F39A mutation (the outer ring) decreases the affinity of rA_{18} to Hfq by 3-4 fold. Hfq binding affinity to rA_{18} is recovered by the F39A/L12F double mutation. The results are consistent with a Hfq binding site for an A-rich sequence that involves the ring of aromatic residues at the radius of position 39.