Supporting Information

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SI Materials and Methods

Plasmid Construction. To construct P_L-*lacI*, the *luc* gene of pZE12-*luc* (1) was replaced by the *lacI*^{q} gene at the KpnI and XbaI sites. To construct P_L-*ryhB*, the *luc* gene of pJV107-8 carrying the *ryhB* gene (2) was digested out using KpnI and XbaI. Thereafter, the sites were filled in using the Klenow fragment of DNA polymerase and ligated.

Primer Extension Assays. Total RNA was extracted using the TriPure reagent (Roche). RNA samples (30 μg) were annealed to the corresponding end-labeled primers (70 °C for 10 min, followed by incubation for 20 min at 42 °C and 10 min at room temperature) and then subjected to primer extension (at 42 °C for 45 min) with 1 unit of AMV-RT (Promega or Roche Diagnostics) and dNTPs (0.5 mM each). The extension products were separated on 6% sequencing gels, alongside with sequencing reactions. The gene-specific primers used were *sdhC* (5′-TGT AGG TCC AGA TTA ACA GGT C-3′), *nuoA* (5′-TTC AGT GGA TGT TGA CAT ACT C-3′), *fdoG* (5′-GCC TTC TGC TGA CCT GC-3′), *sodA* (5′-GGG ATG GCA GGG TAT AG-3′), *sodB* (5′-GTA GTG CAG GTA ATT CG-3′), and 5S (5′-GAG ACC CCA CAC TAC CAT C-3′).

^{32}P-Labeled RNA Synthesis. DNA templates that carry the promoter sequence recognized by T7 RNAP were amplified from K12 DNA by PCR using the following specific primers: RyhB (#678 #567) 5′-CGA AAT TAA TAC GAC TCA CTA TAG GGA CAG GCG ATC AGG AAG ACC CTC GC and 5′-AAA AAA AAA GCC AGC ACC CGG; OxyS (#689 #690) 5′-CGA AAT TAA TAC GAC TCA CTA TAG GGA CAG GAA ACG GAG CGG CAC and 5′-GCG GAT CCT GGA GAT CC; MicA (#1682 #1683) 5′-CGA AAT TAA TAC GA CTC ACT ATA GGG ACA GGA AAG ACG CGC ATT TGT TAT C and 5′-GAA AAA GGC CAC TCG TGA; *sodA* (#1764 #1765) 5′-CGA AAT TAA TAC GAC TCA CTA TAG GGA CAG GGA ACC AAC TGC TTA CGC G and 5′-GCG TTG GCG TTG TTT AC; *sodB* (1762 #1763) 5′-CGA AAT TAA TAC GAC TCA CTA TAG GGA CAG GAT ACG CAC AAT AAG GCT ATT GTA C and 5′-TGG TGC TTG CCG TAG TG; *fdoG* (# 1766 #1767) 5′-CGA AAT TAA TAC GAC TCA CTA TAG GGA CAG GGA GCC AAT TCT GGA CCT TTG and 5′-CAG TTT ATA CGT CCG GGT TTC; *nuoA* (#1768 #1769) 5′-CGA AAT TAA TAC GAC TCA CTA TAG GGA CAG GGC TGT ATA AAA GAA TTT CTA CAG TGA TTG and 5′-CGC CTA CCA GCA TCA GG. Transcription mixtures contained 100–200 ng PCR-generated DNA fragment, 40 mM Tris·HCl (pH 7.9), 2 mM spermidine, 20 mM DTT, 6 mM $MgCl_2$, 20 units of RNase inhibitor (RNasin, Promega), 500 mM of each ATP, GTP, CTP, 40 mM UTP, 10 μCi [^{32}P]-UTP (specific activity 800 Ci/mmol; Amersham Pharmacia Biotech) and 1.5 units T7 RNAP. Transcription was carried out at 37 °C for 2 h. RNA was extracted by phenol/chloroform and then precipitated by ethanol and 1 M NH_4-acetate.

RelA Purification. Cultures of A5039 or A5309 *hfq1*::Ω cells carrying the pQE30-*relA* plasmid (kindly provided by Gad Glaser, Hebrew University, Jerusalem, Israel) were grown in LB medium. At OD_{600} of 0.5 isopropyl-β-D-thio-galactoside was added to a final concentration of 1 mM for 2 h. The cells were harvested and sonicated in 50 mM NaCl, 20 mM NaH_2PO_4 (pH 7.4), and 10 mM imidazole. His-RelA was purified from the lysates using a nickel-loaded HiTrap Chelating HP 1 mL column and then transferred into RelA buffer [50 mM Tris-acetate at pH 8.5, 10 mM potassium phosphate buffer at pH 8.5, 10 mM EDTA, 1 mM DTT, and 25% (vol/vol) glycerol] using a HiTrap desalting 5-mL column in the Akta prime instrument (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Gel Mobility Shift Assays. Binding mixtures of 10 μL contained 1 nM of labeled RNA, Hfq, RelA, or BSA at the indicated concentrations and binding buffer C. Mixtures were incubated at 22 °C for 10 min, and the binding products were separated by 4% native gels.

Assay Buffers. Binding buffer A [50 mM Tris-acetate at pH 8.0, 15 mM $Mg(Ac)_2$, 60 mM KAc, 30 mM NH_4Ac, 1 mM DTT, 0.2 mM EDTA] and binding buffer C (50 mM Hepes at pH 7.5, 10 mM $MgCl_2$, 100 mM NH_4Cl, and 1.5 mM DTT) were used.

1. Lutz R, Bujard H (1997) Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res* 25:1203–1210.

2. Urban JH, Vogel J (2007) Translational control and target recognition by *Escherichia coli* small RNAs *in vivo*. *Nucleic Acids Res* 35:1018–1037.

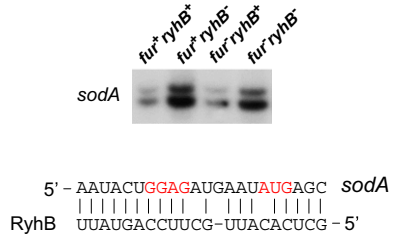

Fig. S1. RyhB regulation of *sodA* mRNA. (*A*) The ribosome-binding region of *sodA* mRNA is complementary to the core sequence of RyhB. Depicted are the core sequence of RyhB (nucleotide position 44–64) and the ribosome binding region of *sodA* mRNA. *sodA* and RyhB complementary nucleotides are indicated by vertical lines. The Shaine–Dalgarno sequence and the AUG are in red. (*B*) RyhB regulation of *sodA* is independent of *fur*. Cultures of *ryhB*$^+$, *ryhB*$^-$, *ryhB*$^+$*fur*$^-$, and *ryhB*$^-$*fur*$^-$ were grown aerated in LB medium at 37 °C to OD_{600} of 0.3 at which they were exposed to the iron chelator 2,2′-dipyridyl (200 μM) for 30 min. Thereafter, total RNA was extracted and subjected to primer extension using an *sodA*-specific primer.

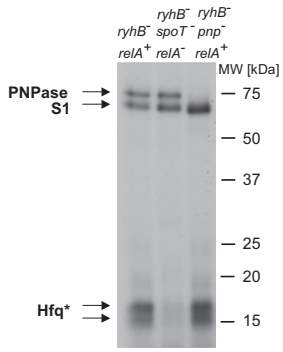

Fig. S2. RelA affects Hfq binding to RyhB in polysomal fractions extracted from cultures grown under conditions of limiting iron. UV cross-linking of labeled RyhB incubated with ribosomes extracted from the strains *relA*$^{+}$, *relA*$^{-}$, and *relA*$^{+}$*pnp*$^{-}$ as indicated. The proteins were analyzed by SDS/PAGE as in Fig. 3*A*. Arrows indicate Hfq, S1, and PNPase proteins. Hfq protein bound to residues of labeled RyhB (Hfq*).

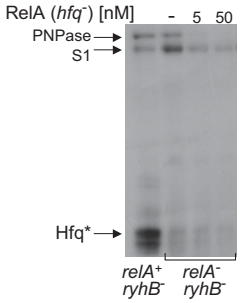

Fig. S3. The addition of purified RelA failed to complement *relA*$^{-}$ polysomal fractions. UV cross-linking of labeled RyhB incubated with ribosomes (22 °C for 15 min.) extracted from *relA*$^{+}$*ryhB*$^{-}$ or *relA*$^{-}$*spoT*$^{-}$*ryhB*$^{-}$. Samples of *relA*$^{-}$*spoT*$^{-}$*ryhB*$^{-}$ were supplemented with 5 nM or 50 nM of RelA purified from *hfq*$^{-}$. Proteins covalently bound to residues of labeled RNA were detected in 15% SDS/PAGE. Arrows indicate Hfq, S1, and PNPase proteins. Hfq protein bound to residues of labeled RyhB (Hfq*). The strain *relA*$^{-}$ is also *spoT*$^{-}$.

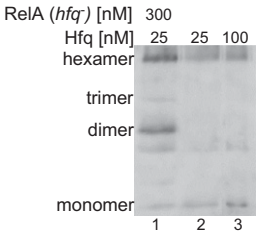

Fig. S4. In vitro oligomerization of Hfq as a function of its concentration. Low and high concentration of Hfq were incubated without or with RelA (where indicated) for 15 min at 22 °C. Thereafter, the proteins were cross-linked using 0.4% freshly diluted gluteraldehyde for 1.5 min. Cross-linking was stopped with freshly made glycine (200 nM). The proteins were boiled in loading buffer, and equal amounts were loaded in 15% SDS/PAGE. Hfq was detected by Western blotting using α-Hfq. Measurement of the ratio of hexamer to dimer (when present) to monomer shows a proportion of 0.5, 0.33, and 0.04, respectively (lane 1); 0.62 and 0.25 (lane 2); and 0.66 and 0.25 (lane 3).

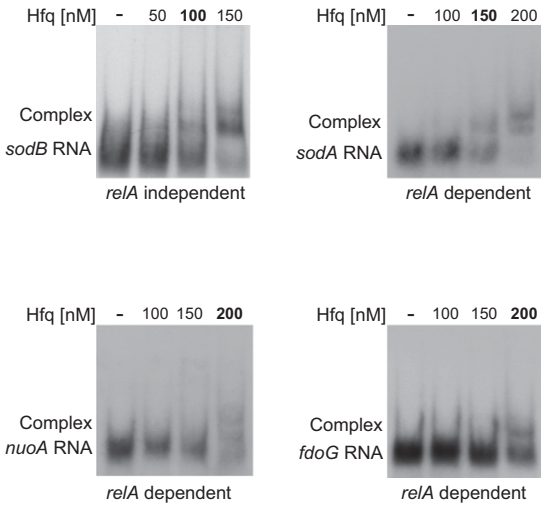

Fig. S5. *sodB*, the RelA-independent RhyB target exhibits a higher affinity for Hfq than the RelA-dependent targets. Gel mobility shift of labeled *sodA*, *sodB*, *nuoA*, or *fdoG* RNAs incubated with increasing amounts of Hfq. Bound and unbound labeled mRNAs are indicated. Hfq concentration at which the shift is clearly detected is marked by boldface type.

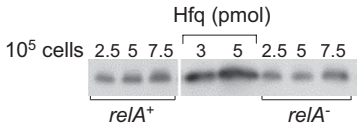

Fig. S6. Estimation of the number of Hfq monomers in vivo. Cultures of *relA*+ and *relA*⁻*spoT*⁻ were grown in LB to OD_{600} of 2.0 at which samples of 800 μL were collected, and their pellets were suspended in 80 μL of 1× loading buffer and boiled. The proteins were separated in 15% SDS/PAGE, and Hfq was detected by Western blotting using α-Hfq. Samples of purified Hfq (3 and 5 pmol) were loaded as a reference. We estimate that *relA*+ and *relA*⁻*spoT*⁻ carry ~5,000 and ~4,000 hexamers per cell, respectively.