Supporting Information

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SI Materials and Methods.

Media and Growth Conditions. Cells were grown aerobically at 37 °C in LB medium supplemented with indicated compounds. Antibiotics were used at the following concentrations when needed: kanamycin (15 μg/mL) and ampicillin (50 μg/mL). Bacterial growth was monitored by determining the optical density at 600 nm.

Construction of Plasmids. Chromosomal or plasmid DNAs were used as templates for PCR. A DNA fragment containing the *araC-araBAD* promoter region up to +1 relative to the transcription start site was amplified, digested with *Eco*RI and *Sal*I, and cloned into the low copy number plasmid pMW218 to obtain pAraS. Similarly, a DNA fragment containing the *araC-araBAD* promoter region was amplified, digested with *Eco*RI and *Xba*I, and cloned into pMW218 to obtain pAraX. Plasmids pAraS and pAraX were used as vectors to construct a series of plasmids expressing sRNAs and their variants. A DNA fragment containing the full-length *sgrS* was amplified, digested with *Sal*I and *Hin*dIII, and cloned into pAraS to obtain pSgrS. A DNA fragment containing the truncated *sgrS-S* was amplified, digested with *Xba*I and *Hin*dIII, and cloned into pAraX to obtain pSgrS-S. DNA fragments containing the genes for a series of SgrS variants were amplified, digested with *Sal*I and *Hin*dIII, and cloned into pAraS to obtain the corresponding plasmids. DNA fragments containing the genes for other sRNAs and their variants were amplified, digested with *Xba*I and *Hin*dIII, and cloned into pAraX to obtain the corresponding plasmids. In all cases, the region from the transcription start to a few bases downstream from the last T of the terminator polyT was amplified. Plasmids pRyhB and pRyhB-4U were digested with *Eco*RI and *Hin*dIII. The resulting fragments were cloned into the high copy number plasmid pTWV228 to obtain pT-RyhB and pT-RyhB-4U, respectively. The nucleotide sequences of all constructs were verified by DNA sequencing.

DNA Probes for Northern Blotting. The following digoxigenin (DIG)-labeled DNA probes were prepared by PCR using DIG-dUTP: 305-bp fragment corresponding to the 5′ region of *ptsG* (*ptsG* probe); 150-bp fragment containing the 5′ portion (+1 to +150) of SgrS (SgrS probe 1); 60-bp fragment containing the 3′ portion (+168 to +227) of SgrS (SgrS probe 2); 210-bp fragment corresponding to the 5′ region of *sodB* (*sodB* probe); 87-bp fragment corresponding to the *ryhB* (RyhB probe); 235-bp fragment corresponding to the 5′ region of *ompA* (*ompA* probe); 220-bp fragment corresponding to the 5′ region of *ompF* (*ompF* probe); 85-bp fragment corresponding to the *micF* (MicF probe). A 5′ end DIG-labeled synthetic DNA oligonucleotide corresponding to antisense sequence of the 1–50 nt of MicA (Rikaken Co. Ltd.) was used as the MicA probe. RA Low II Easy Load (BioDynamics Laboratory Inc.) was used as RNA marker when indicated.

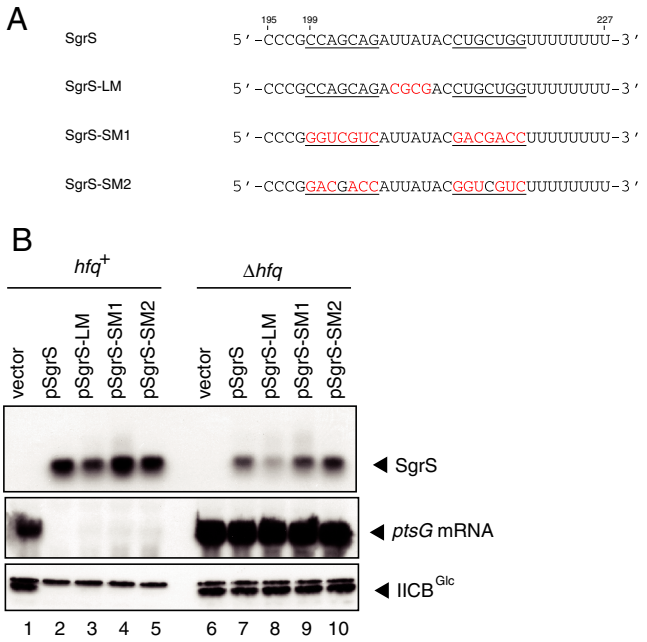

Fig. S1. Effects of mutations in the terminator hairpin on SgrS function. (*A*) Nucleotide sequences around the terminator of SgrS variants. Nucleotides changed from the wild-type SgrS sequence are shown in red. The inverted repeat sequences are underlined. (*B*) Effects of mutations on the expression and function of SgrS. IT1568 and TM589 cells harboring indicated plasmids were grown in LB medium. At A_{600} = 0.6, total RNAs were prepared, and 0.25 or 7 μg of each RNA sample was subjected to Northern blot analysis using the SgrS probe 1 or the *ptsG* probe. Total proteins were also prepared and samples equivalent to 0.025 A_{600} units were subjected to Western blot analysis using anti-IIB antibodies.

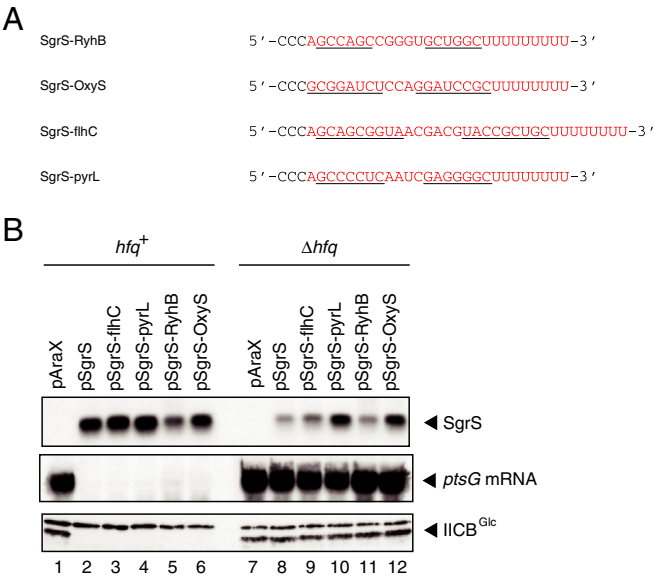

Fig. S2. Effects of foreign terminators on SgrS function. (*A*) Nucleotide sequences around the terminator of SgrS variants. Nucleotides corresponding to foreign terminators are shown in red. The inverted repeat sequences are underlined. (*B*) Expression of chimeric SgrS variants and their effect on *ptsG* expression. IT1568 and TM589 cells harboring indicated plasmids were grown in LB medium. At $A_{600} = 0.6$, total RNAs were prepared and 0.25 or 7 μg of each RNA sample was subjected to Northern blot analysis using the SgrS probe 1 or the *ptsG* probe. Total proteins were also prepared and samples equivalent to 0.025 A_{600} units were subjected to Western blot analysis using anti-IIB antibodies.

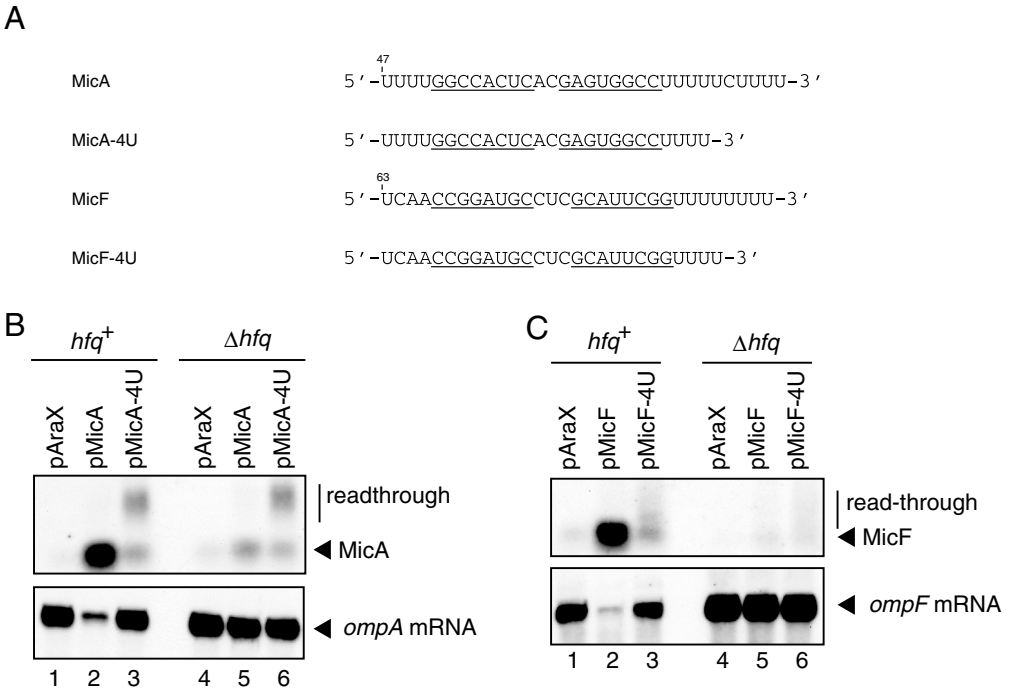

Fig. S3. Effects of polyU tail shortening of on the expression and function of MicA and MicF. (*A*) Nucleotide sequences around the terminator. The inverted repeat sequences are underlined. (*B*) Expression and function of MicA and MicA-4U. IT1568 and TM589 cells harboring indicated plasmids were grown in LB medium. At $A_{600} = 0.6$, total RNAs were prepared and 1 μg of each RNA sample was subjected to Northern blot analysis using the MicA and *ompA* probes. (*C*) Expression and function of MicF and MicF-4U. IT1568 and TM589 cells harboring indicated plasmids were grown in LB medium. At $A_{600} = 0.6$, total RNAs were prepared and 1 μg of each RNA sample was subjected to Northern blot analysis using the MicF and *ompF* probes.

Table S1. Nucleotide sequences around the rho-independent terminators of sRNA genes used in this study

sgrS/sgrS-S	5′-CCCGCCAGCAGATTATACCTGCTGGTTTTTTTTAT-3′
sgrS-LM	5′-CCCGCCAGCAGACGCGACCTGCTGGTTTTTTTTAT-3′
sgrS-SM1	5′-CCCGGGTCGTCATTATACGACGACCTTTTTTTTAT-3′
sgrS-SM2	5′-CCCGGACGACCATTATACGGTCGTCTTTTTTTTAT-3′
sgrS-7T	5′-CCCGCCAGCAGATTATACCTGCTGGTTTTTTTAT-3′
sgrS-6T	5′-CCCGCCAGCAGATTATACCTGCTGGTTTTTTAT-3′
sgrS-5T	5′-CCCGCCAGCAGATTATACCTGCTGGTTTTTAT-3′
sgrS-4T	5′-CCCGCCAGCAGATTATACCTGCTGGTTTTAT-3′
sgrS-LS8T	5′-CCCGCCAGCAGCCCCATTATACGGGGCTGCTGGTTTTTTTTAT-3′
sgrS-LS4T	5′-CCCGCCAGCAGCCCCATTATACGGGGCTGCTGGTTTTAT-3′
sgrS-ryhB	5′-CCCAGCCAGCCGGGTGCTGGCTTTTTTTTGA-3′
sgrS-oxyS	5′-CCCGCGGATCTCCAGGATCCGCTTTTTTTTGC-3′
sgrS-flhC	5′-CCCAGCAGCGGTAACGACGTACCGCTGCTTTTTTTTGC-3′
sgrS-pyrL	5′-CCCAGCCCCTCAATCGAGGGGCTTTTTTTTGC-3′
ryhB	5′-CTTAGCCAGCCGGGTGCTGGCTTTTTTTTGA-3′
ryhB-4T	5′-CTTAGCCAGCCGGGTGCTGGCTTTTGA-3′
micA	5′-TTTTGGCCACTCACGAGTGGCCTTTTTCTTTTCTGTCAGG-3′
micA-4T	5′-TTTTGGCCACTCACGAGTGGCCTTTTCAGG-3′
micF	5′-TCAACCGGATGCCTCGCATTCGGTTTTTTTTACCC-3′
micF-4T	5′-TCAACCGGATGCCTCGCATTCGGTTTTACCC-3′

Table S2. PolyT tails of rho-independent terminators of *Escherichia coli* Hfq-binding sRNAs

Name of sRNA gene	Sequence of polyT tail of rho-independent terminator
ryhB	TTTTTTTTT (9T)
micC	TTTTTTTTT (9T)
sgrS	TTTTTTTATT (8TA2T)
rseX	TTTTTTTTAT (8TAT)
oxyS	TTTTTTTT (8T)
omrA	TTTTTTTT (8T)
omrB	TTTTTTTT (8T)
micF	TTTTTTTT (8T)
gcvB	TTTTTTTT (8T)
rprA	TTTTTTTT (8T)
mgrR	TTTTTTTT (8T)
dsrA	TTTTTTTATT (7TA2T)
glmZ	TTTTTTTAT (7TAT)
chiX (micM)	TTTTTTT (7T)
spf (SpoT42)	TTTTTTATT (6TA2T)
rybB	TTTTTTGTT (6TG2T)
arcZ	TTTTTT (6T)
micA	TTTTTCTTTT (5TC4T)
rydC	TTTTCTTT (4TC3T)
cyaR	TTATTTTTT (2TA6T)

The sequences were taken from Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/).

Table S3. Bacterial strains and plasmids used in this study

Strain/plasmid	Relevant genotype and property	Source/reference
Strain		
IT1568	W3110*mlc*	laboratory stock
TM589	W3110*mlc* Δ *hfq*	Morita et al. (1
TM542	W3110*mlc* Δ *(sgrR-sgrS)*	Kawamoto et al. 2
TM615	W3110mlc *hfq-FLAG-cat*	Morita et al. 1
TM771	W3110*mlc* Δ *(sgrR-sgrS) hfq-FLAG-cat*	this study
Plasmid		
pMW218	Low copy number plasmid vector	NIPPON GENE
pAraS	Derivative of pMW218 carrying the *araC-PBAD*	this study
pAraX	Derivative of pMW218 carrying the *araC-PBAD*	this study
pSgrS	Derivative of pAraS carrying the *sgrS*	this study
pSgrS-S	Derivative of pAraX carrying the *sgrS-S*	this study
pSgrS-LM	Derivative of pAraS carrying the *sgrS-LM*	this study
pSgrS-SM1	Derivative of pAraS carrying the *sgrS-SM1*	this study
pSgrS-SM2	Derivative of pAraS carrying the *sgrS-SM2*	this study
pSgrS-7U	Derivative of pAraS carrying the *sgrS-7T*	this study
pSgrS-6U	Derivative of pAraS carrying the *sgrS-6T*	this study
pSgrS-5U	Derivative of pAraS carrying the *sgrS-5T*	this study
pSgrS-4U	Derivative of pAraS carrying the *sgrS-4T*	this study
pSgrS-LS8U	Derivative of pAraS carrying the *sgrS-LS8T*	this study
pSgrS-LS4U	Derivative of pAraS carrying the *sgrS-LS4T*	this study
pSgrS-RyhB	Derivative of pAraS carrying the *sgrS-ryhB*	this study
pSgrS-OxyS	Derivative of pAraS carrying the *sgrS-oxyS*	this study
pSgrS-flhC	Derivative of pAraS carrying the *sgrS-flhC*	this study
pSgrS-pyrL	Derivative of pAraS carrying the *sgrS-pyrL*	this study
pRyhB	Derivative of pAraX carrying the *ryhB*	this study
pRyhB-4U	Derivative of pAraX carrying the *ryhB-4T*	this study
pMicA	Derivative of pAraX carrying the *micA*	this study
pMicA-4U	Derivative of pAraX carrying the *micA-4T*	this study
pMicF	Derivative of pAraX carrying the *micF*	this study
pMicF-4U	Derivative of pAraX carrying the *micF-4T*	this study
pTWV228	High copy number plasmid vector	TAKARA
pT-RyhB	Derivative of pTWV228 carrying the *araC-PBAD-ryhB*	this study
pT-RyhB-4U	Derivative of pTWV228 carrying the *araC-PBAD-ryhB-4T*	this study

1. Morita T, Maki K, Aiba H (2005) RNase E-based ribonucleoprotein complexes: Mechanical basis of mRNA destabilization mediated by bacterial noncoding RNAs. *Genes Dev* 19:2176–2186.
2. Kawamoto H, et al. (2005) Implication of membrane localization of target mRNA in the action of a small RNA: Mechanism of post-transcriptional regulation of glucose transporter in *Escherichia coli*. *Genes Dev* 19:328–338.

Table S4. DNA primers used for construction of plasmids

Primer	Sequence	Plasmid
926	CCCCCGAATTCCTGATTCGTTACCAA	pAraS, pAraX (F)
927	CCCCGTCGACGGTATGGAGAAACAGTAG	pAraS (R)
975	CGCGTCTAGAAACAGTAGAGAGTT	pAraX (R)
928	CCCCGTCGACGATGAAGCAAGGGGG	pSgrS, pSgrS-LM, -SM1, -SM2, -4U, -5U, -6U, -7U, -LS8U, -LS4U, -RyhB, -OxyS,-flhC, -pyrL (F)
930	CCCAAGCTTATAAAAAAAACCAGCAGG	pSgrS (R)
1,127	GCGCTCTAGATAGTGTGACTGAGTATTGGT	pSgrS-S (F)
929	CCCAAGCTTGATAGCCATCAAACAGC	pSgrS-S ((R)
1,236	CCCAAGCTTATAAAAAAAACCAGCAGGTCGCGTCTGCTGGCGGGTGATTTTACAC	pSgrS-LM (R)
HO14	CCCAAGCTTATAAAAAAAAGGTCGTCGTATAATGACGACCCGGGTGATTTTACAC	pSgrS-SM1 (R)
HO15	CCCAAGCTTATAAAAAAAAGACGACCGTATAATGGTCGTCCGGGTGATTTTACAC	pSgrS-SM2 (R)
HO17	CCCAAGCTTATAAAACCAGCAGGTATAATCTGCT	pSgrS-4U (R)
HO18	CCCAAGCTTATAAAAACCAGCAGGTATAATCTGCT	pSgrS-5U (R)
HO19	CCCAAGCTTATAAAAAACCAGCAGGTATAATCTGCT	pSgrS-6U (R)
HO20	CCCAAGCTTATAAAAAAACCAGCAGGTATAATCTGCT	pSgrS-7U (R)
1,118	CCCAAGCTTATAAAAAAAACCAGCAGCCCCGTATAATGGGGCTGCTGGCGGGTGATTTTAC	pSgrS-LS8U (R)
1,120	CCCAAGCTTATAAAACCAGCAGCCCCGTATAATGGGGCTGCTGGCGGGTGATTTTAC	pSgrS-LS4U (R)
1,176	CCCAAGCTTACGAAAGATCAAAAAAAAAGCCAGCACCCGGCTGGCTGGGTGATTTTACACCAATAC	pSgrS-RyhB (R)
1,177	CCCAAGCTTTTTTTATGGCAAAAAAAAGCGGATCCTGGAGATCCGCGGGTGATTTTACACCAATAC	pSgrS-OxyS (R)
1,172	CCCAAGCTTCGATTGGGGCAAAAAAAAGCAGCGGTACGTCGTTACCGCTGCTGGGTGATTTTACACCAATAC	pSgrS-flhC (R)
1,171	CCCAAGCTTACGCCTGGGCAAAAAAAAGCCCCTCGATTGAGGGGCTGGGTGATTTTACACCAATAC	pSgrS-pyrL (R)
1,144	GCGCTCTAGATAGCGATCAGGAAGACCCTC	pRyhB, pRyhB-4U (F)
1,145	CCCAAGCTTTGAGAACGAAAGATCAAAAA	pRyhB (R)
1,152	CCCAAGCTTTGAGAACGAAAGATCAAAAGCCAGCACCCGGCTGG	pRyhB-4U (R)
1,148	GCGCTCTAGATAGAAAGACGCGCATTTGTT	pMicA, pMicA-4U (F)
1,149	CCCAAGCTTTGGAAAAACACGCCTGACAG	pMicA (R)
1,154	CCCAAGCTTTGGAAAAACACGCCTGAAAAGGCCACTCGTGAGTGG	pMicA-4U (R)
1,150	GCGCTCTAGATAGCTATCATCATTAACTTT	pMicF, pMicF-4U (F)
1,151	CCCAAGCTTAAAAGTGTGTAAAGAAGGGT	pMicF (R)
1,155	CCCAAGCTTAAAAGTGTGTAAAGAAGGGTAAAACCGAATGCGAGGCATC	pMicF-4U (R)