Supporting Information

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SI Materials and Methods

RNA sequencing. Identity of RNA component in T7 gp5.5–HNS complex was determined by sequence analysis as previously described (1), with some modification. Briefly, the RNA component was isolated from purified T7 gp5.5–HNS complex by phenol-chloroform extraction. After ligation to adaptors, RNA was amplified by reverse transcriptase. Reaction products were amplified by PCR and inserted into HindIII site in pUC19. DNA sequence analysis of the insert in the plasmid provided identity of RNA component.

RNA Primed DNA Synthesis. M13 ssDNA (8 nM) was incubated with 40 mM Tris·HCl (pH 7.5), 10 mM DTT, 10 mM $MgCl_2$, 50 mM potassium glutamate, 600 μM dNTP containing [^{3}H] dGTP, 10 μM RNA primer (ACCA or $tRNA^{Arg}_{ACG}$), 150 nM 56-kDa gp4, and 100-nM T7 DNA polymerase at 37 °C. dGMP incorporated into the DNA in 5-μL reaction solution was counted at various time.

Endonuclease Assays. Endonucleolytic digestion of the supercoiled plasmid pUC(AT) was performed as previously described (2). The dsDNA with a 5′-ssDNA tail (flap) structure was prepared by annealing a 36-mer (5′-T_{16} GAG GCA GTT CGA GTG GTG AG-3′) and a 16-mer (5′-CAG TGC TCG TGA ACT C-3′) to a 36-mer (5′-CTC ACC ACT CGA ACT GCC TCG AGT TCA CGA GCA CTG-3′). One of 36-mer was [5′-^{32}P] labeled. The DNA substrate (50 nM) was incubated with various amount of wild-type or altered gene 3 endonuclease at 37 °C for 20 min in a buffer containing 50 mM Tris·HCl (pH 7.9), 10 mM $MgCl_2$, 1 mM DTT, and 100 mM NaCl. Reaction products were separated on 15% denaturing polyacrylamide Tris-borate-EDTA (TBE) gel containing 7 M urea, and radioactivity in products was measured using a Fuji BAS 1000 Bioimaging analyzer.

Substrates for Mobility Shift Assay. The Y-shaped dsDNA substrate was prepared by annealing a [5′-^{32}P]-labeled 75-mer (5′-CGC CGG GTA CCG AGC TCG AAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC ATG CCT_{19}-3′) to a 95-mer (5′-T_{39} GGC ATG TCA CGA CGT TGT AAA ACG ACG GCC AGT GAA TTC GAG CTC GGT ACC CGG CG-3′). The ssDNA substrate was the [5′-^{32}P]-labeled 75-mer only, and the dsDNA substrate with an ssDNA tail was prepared by annealing the [5′-^{32}P]-labeled 75-mer (5′-CGC CGG GTA CCG AGC TCG AAT TCA CTG GCC GTC GTT TTA CAA CGT CGT GAC ATG CCT_{19}-3′) to a 55-mer (5′-GC ATG TCA CGA CGT TGT AAA ACG ACG GCC AGT GAA TTC GAG CTC GGT ACC CGG CG-3′).

DNA Unwinding Assay. Assays were performed as described previously (3), except that various amounts of gp4 were used and H-NS or H-NS/gp5.5/transfer RNA (tRNA) complex was added in some reactions.

DNA Synthesis Assay. To measure the effect of H-NS on DNA synthesis, the mixture containing 40 mM Tris·HCl (pH 7.5), 10 mM DTT, 10 mM $MgCl_2$, 50 mM potassium glutamate, 600 μM dNTP containing [^{3}H] dGTP, 8 nM M13 ssDNA annealed with primer or M13 dsDNA, 100 nM T7 DNA polymerase. The 56-kDa gp4 (150 nM) and/or designated amount of H-NS or H-NS/gp5.5/tRNA complex were added in indicated reactions.

1. Lau NC, Lim LP, Weinstein EG, Bartel DP (2001) An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science* 294:858–862.
2. Lee SJ, Chowdhury K, Tabor S, Richardson CC (2009) Rescue of bacteriophage T7 DNA polymerase of low processivity by suppressor mutations affecting gene 3 endonuclease. *J Virol* 83:8418–8427.
3. Zhu B, Lee SJ, Richardson CC (2009) An in trans interaction at the interface of the helicase and primase domains of the hexameric gene 4 protein of bacteriophage T7 modulates their activities. *J Biol Chem* 284:23842–23851.

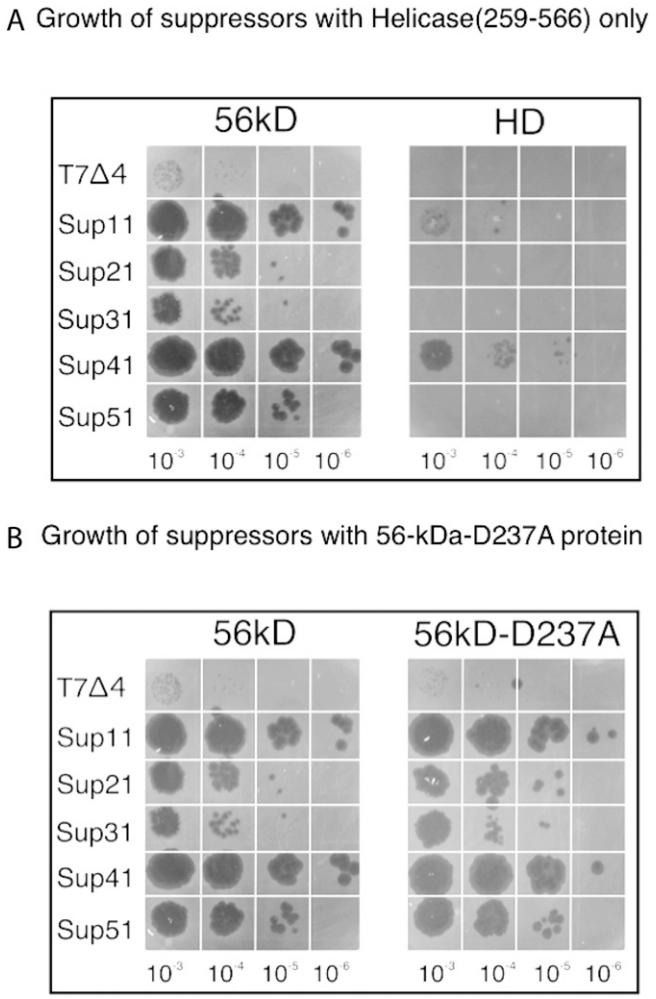


Fig. S1. T7 phage suppressors require more than helicase domain but not an active site of primase. (*A*) Infection of wild-type and suppressor T7Δ4 phages on *Escherichia coli* expressing 56-kDa gp4 or helicase domain only. (*B*) Infection of wild-type and suppressor T7Δ4 phages on *E. coli* expressing 56-kDa gp4 or 56-kDa gp4 containing substitution of Ala for Asp237 that disrupts the primase active site.

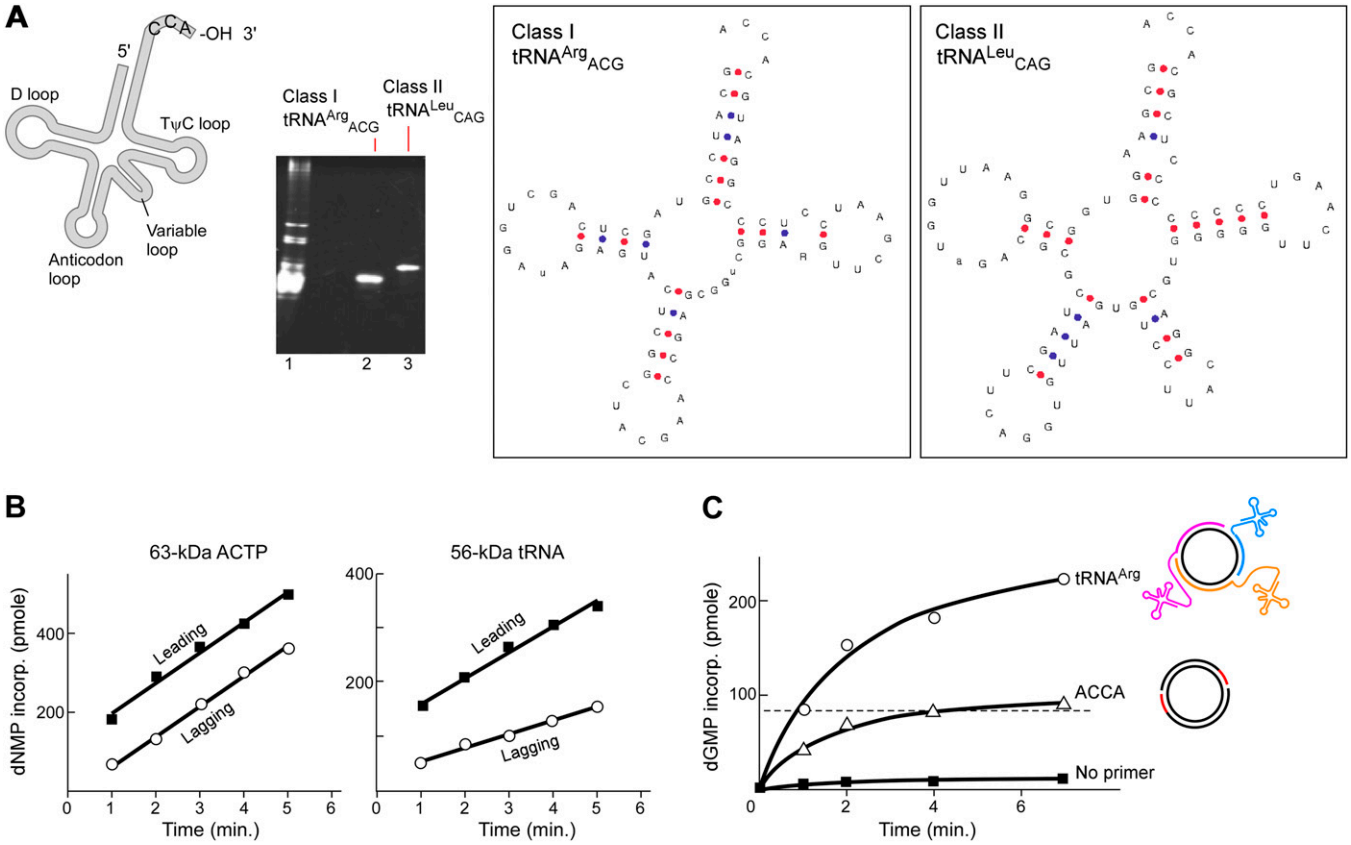


Fig. S2. tRNA priming. (*A*) Common structure of tRNA consists of a D-loop, an anticodon loop, a variable loop, a TψC loop, and an acceptor stem ended by the CCA terminus. The size of variable loop differentiates two classes of tRNA. Two representatives of each class, $tRNA^{Arg}_{ACG}$ (class I) and $tRNA^{Leu}_{CAG}$ (class II), were purified and used in following assays. tRNA secondary structures were from Genomic tRNA Database (http://gtrnadb.ucsc.edu/). On the *Inset* gel, lane 1, *E. coli* total tRNA; lane 2, purified $tRNA^{Arg}_{ACG}$; lane 3, purified $tRNA^{Leu}_{CAG}$. (*B*) Coordinated DNA synthesis by T7 replisome. *Left*: Primer is synthesized by T7 primase using ATP and CTP. *Right*: Primer is $tRNA^{Arg}_{ACG}$. Coordinated DNA synthesis was carried out as previously reported (1, 2), except that the 63-kDa gp4 and ATP/CTP might be replaced by 56-kDa gp4 and 20 μM $tRNA^{Arg}_{ACG}$. (*C*) DNA synthesis on M13 ssDNA template mediated by gp5/trx and 56-kDa gp4 in the absence of primer or in the presence of ACCA or $tRNA^{Arg}_{ACG}$. A dashed line indicates the completion of the complementary strand of M13 DNA in this experiment.

1. Lee J, Chastain PD, 2nd, Kusakabe T, Griffith JD, Richardson CC (1998) Coordinated leading and lagging strand DNA synthesis on a minicircular template. *Mol Cell* 1:1001–1010.
2. Zhu B, Lee SJ, Richardson CC (2010) Direct role for the RNA polymerase domain of T7 primase in primer delivery. *Proc Natl Acad Sci USA* 107:9099–9104.

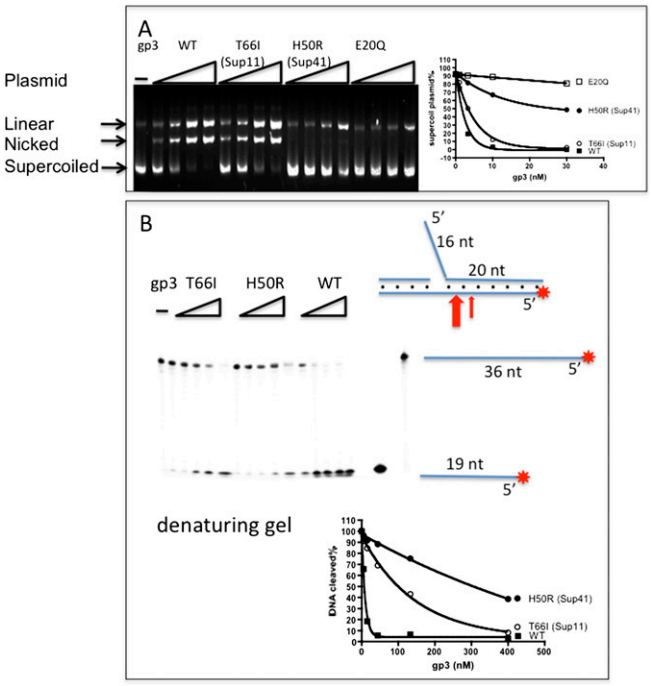
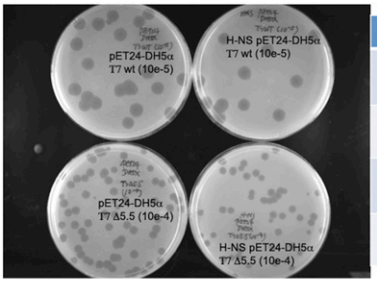
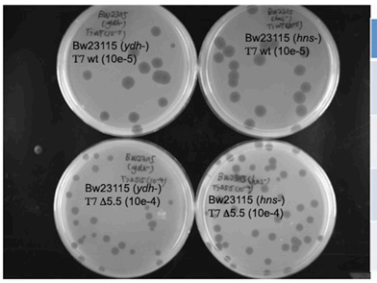


Fig. S3. Activities of gp3 variants found in T7 suppressor mutants. (*A*) Supercoiled plasmid pUC(AT) (30 nM) containing a sequence of ~20 bp of alternating AT sequence was incubated with increasing amounts of the indicated gp3 (1, 3, 10, and 30 nM) at 37 °C for 20 min. Reaction products were analyzed on 1% agarose gel stained by ethidium bromide. Initial supercoiled substrate and linear or nicked products are indicated on the left side of the gel. Decreasing of supercoiled plasmid catalyzed by each gp3 variant was quantified and plotted against gp3 concentration. Gp3 E20Q mutant was applied as a negative control. (*B*) A 36-bp dsDNA substrate containing a 16-nt 5′-single-stranded tail (flap) (*Inset*) was incubated with various amounts of wild-type or gp3 variants, and the products were analyzed on a 15% denaturing TBE gel. The complementary strand to the flap was 5′-labeled with ^{32}P. The same [5′-^{32}P]-labeled 36-nt ssDNA and a 19-nt ssDNA (same sequence as the 19 nt from the 5′ terminus of the 36-nt ssDNA) were used as markers. Decreasing of original substrates catalyzed by each gp3 variant was quantified and plotted against gp3 concentration.



	H-NS	gp5.5	EOP	Plq size
pET24-DH5α T7 wt	+	+	1	1
H-NS-pET24-DH5α T7 wt	++	+	0.5	1
pET24-DH5α T7 Δ5.5	+	-	0.4	0.5
H-NS-pET24-DH5α T7 Δ5.5	++	-	0.2	0.4



	H-NS	gp5.5	EOP	Plq size
Bw23115 (*ydh*-) T7 wt	+	+	1	1
Bw23115 (*hns*-) T7 wt	-	+	2	1
Bw23115 (*ydh*-) T7 Δ5.5	+	-	0.3	0.4
Bw23115 (*hns*-) T7 Δ5.5	-	-	0.6	0.5

Fig. S4. Effect of gp5.5 and H-NS level on T7 phage growth. Efficiency of plating (EOP) was measured by plating assays as in the figure. At least two dilutions of each phage were plated on an *E. coli* lawn expressing the genes encoding 63-kDa or 56-kDa gp4. The plaque numbers are proportional to the phage concentrations with a SD less than 5% of the mean. The mean number was derived and normalized against the value obtained with wild-type T7 in the absence of modification on H-NS level. Plaque size is also relative value normalized against the plaque size of wild-type T7 phage without modification of H-NS level. Bw23115 (*ydh*-) is used as an *hns*+ control.

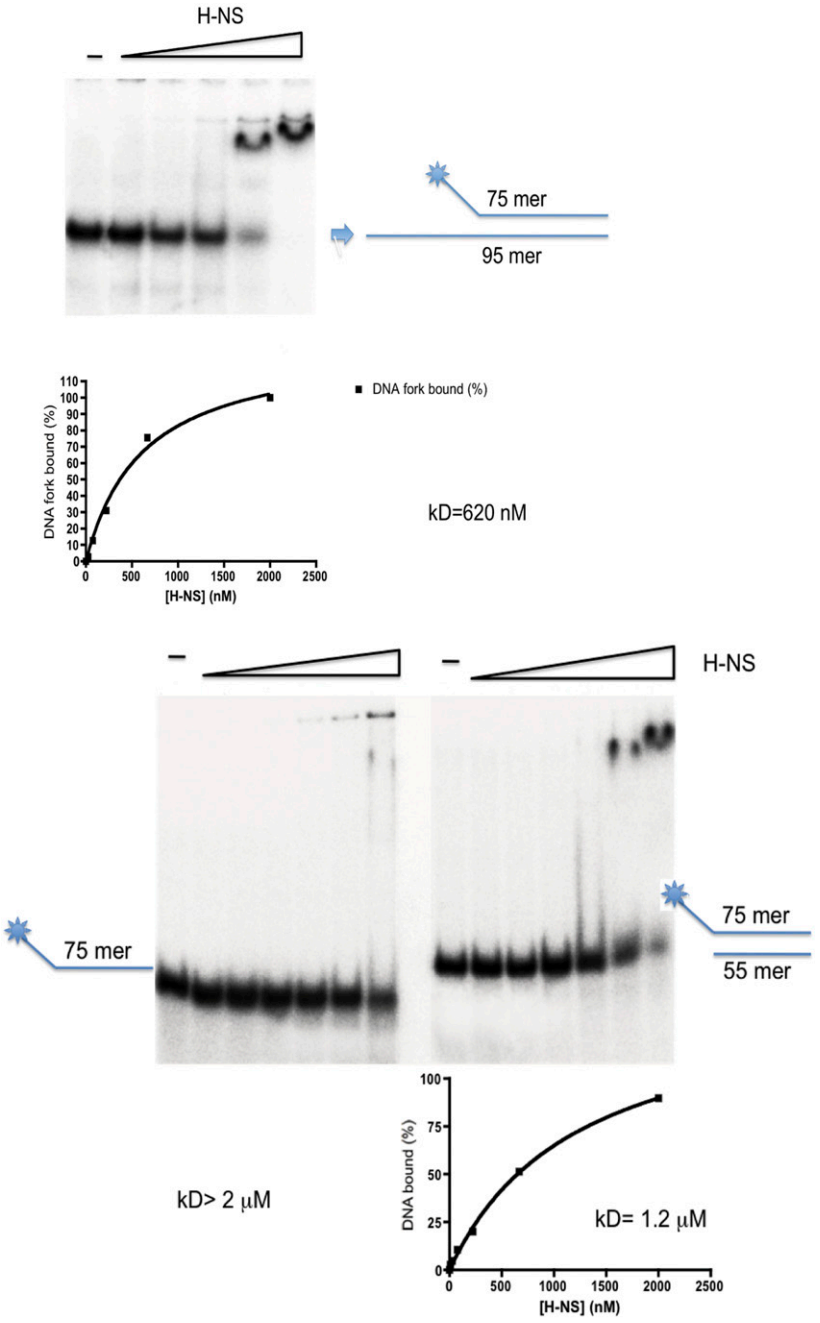


Fig. S5. Binding of H-NS to a Y-shaped dsDNA, an ssDNA, or a dsDNA with ssDNA tail analyzed by mobility shift assay. Substrates preparation is described in *SI Materials and Methods* and reaction conditions are described in *Materials and Methods*.

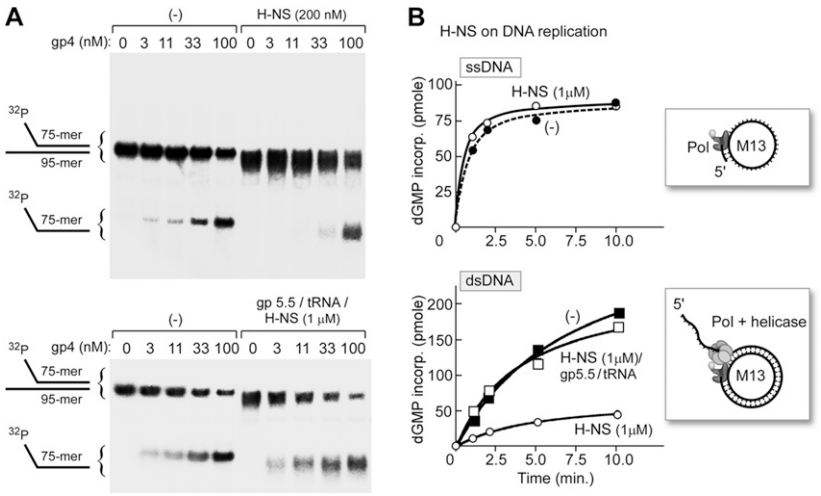


Fig. S6. Inhibitory effect of *E. coli* H-NS on DNA helicase. (*A*) H-NS inhibits DNA unwinding by T7 gp4 helicase. Unwinding of dsDNA by T7 gp4 helicase (33 nM) is inhibited by 200 nM H-NS. H-NS (1 μM) in complex with gp5.5 and tRNA does not show an inhibitory effect. (*B*) DNA synthesis catalyzed by T7 DNA polymerase on circular ssDNA bearing a primer is not affected by 1 μM H-NS. Leading-strand DNA synthesis mediated by T7 DNA polymerase and helicase is inhibited by 1 μM H-NS but not by H-NS in complex with gp5.5 and tRNA.

Table S1. Sequencing data of RNAs in complex with gp5.5 and H-NS

Sequence	Sequence (5′-3′)	Antiparallel sequence (5′-3′)	Identity (sequence in red)
Seq. 1	tggtgggggctatagctcagctgggagagcgcttgcag		1–32 nt of *E. coli* $tRNA^{Ala}_{GGC}$
Seq. 2	tggagcgggaaacgagactcgaactcgcgaccccgaccttggcaag	cttgccaaggtcggggtcgcgagttcgagtctcgtttcccgctcca	31–76 nt of *E. coli* $tRNA^{Gly}_{GCC}$
Seq. 3	tggcgtcccctaggggattcgaacccctgttaccgccgtgaaagggcggtgtcctgggcctctagacgaaggggacgg	ccgtcccttcgtctagaggcccaggacaccgcccttcacggcggtaacaggggttcgaatcccctaggggacgcca	Full length *E. coli* $tRNA^{Glu}_{TTC}$
Seq. 4	caaaagagatcgcgtggaagccctgcctggggttgaagcgttaaaacttaatcaggctagtttgttagtggcgtgtccgtccgcagctggcaagcgaatataaagactgactaagcatgtagtaccgaggatgtaggaatttcggacgcgggttcaactcccgccagctccacca		3′-fragment of *E. coli* tmRNA

Identity of RNAs is based on the sequences shown in red.