Supporting Information

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SI Materials and Methods

Reagents, Strains, and Plasmids. Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl) was from Acumedia, and agar was from Difco. Antibiotics, isopropyl-β-D-thiogalactopyranoside (IPTG) and L-arabinose were from Sigma-Aldrich. Restriction enzymes were from New England Biolabs. Rapid ligation kit was from Roche. The bacterial strains, phages, plasmids, and oligonucleotides used in this study are listed in Table S1.

Plasmid Construction. Plasmid p0.4-CTAP encodes the last 50 nt of gene 0.4 fused, in frame, to a C-terminal tandem affinity purification (CTAP) tag, which encodes a C-terminal calmodulin-binding peptide, a specific TEV protease recognition sequence, and an IgG-binding unit of protein A of *Staphylococcus aureus* (1). This tag was followed by the stop codon of gene 0.4 50 nt downstream and then by thioredoxin A (*trxA*), a positive selection genetic marker for T7 bacteriophage grown on hosts lacking *trxA*. Plasmid p0.4-CTAP was constructed by PCR amplification of phage T7 encoding the CTAP-*trxA* fragment by using primers RK1F and RK1R (Table S1) containing NdeI and XbaI restriction sites, respectively. The PCR fragment was digested with NdeI and XbaI and ligated to a compatibly digested pUC19 plasmid. Plasmid pBAD-0.4 was constructed by PCR amplification of WT phage T7 (Table S1) using primers RK11Fa and RK11Ra (Table S1), containing restriction sites XmaI and XbaI, respectively. The PCR fragment was digested with XmaI and XbaI and consequently ligated to compatibly digested plasmid pBAD18.

Plasmid pUC-0.4 (Table S1) was constructed to delete gene 0.4 in the T7 genome, resulting in a DNA "scar" in the form of flippase recognition target (FRT) sites. Plasmid pUC-ctrl (Table S1) was constructed to insert an identical DNA scar immediately downstream of gene 0.4. Both plasmids encode the *trxA* gene, a positive selection marker for T7 grown on hosts lacking *trxA*, flanked by FRT sites. The *trxA* in the pUC-0.4 and pUC-ctrl plasmids is flanked by 50 bp upstream and downstream of the DNA sequence encoding gene 0.4 or the 50 bp immediately downstream and upstream the stop codon of gene 0.4, respectively. The plasmids were constructed by PCR amplification of T7 phage encoding the *trxA* gene flanked by FRT sites by using primers RK53F and RK53R for the pUC-0.4 plasmid and primers RK53R and RK60F (Table S1) for the pUC-ctrl plasmid, which contained the PvuII restriction sites. The PCR fragment was digested with PvuII and was ligated to a compatibly digested pUC19 plasmid.

Homologous Recombination of Bacteriophage T7. T7 containing gene 0.4 with a CTAP tag was constructed by homologous recombination. Plasmid p0.4-CTAP was transformed into *Escherichia coli* strain NEB5α (Table S1). *E. coli* NEB5α/p0.4-CTAP were aerated overnight in LB supplemented with 100 μg/mL ampicillin at 37 °C. The overnight culture was diluted 1:20 in fresh LB supplemented with 100 μg/mL ampicillin at 42 °C and aerated to an OD_{600} of 0.5. The cells were then infected with WT phage T7 at a multiplicity of infection (MOI) of 0.1. The infected bacteria were aerated at 42 °C until complete clearing of the culture. The obtained lysate was used to infect *E. coli* lacking *trxA* (BW25113Δ*trxA*; Table S1). This procedure selected for phages that had recombined the fragment containing *trxA* into their genome. Strain BW25113Δ*trxA* was aerated overnight in LB supplemented with 25 μg/mL kanamycin at 37 °C. The overnight culture was diluted 1:1 in 3 mL of fresh LB supplemented with 25 μg/mL kanamycin at 37 °C and aerated for 1 h. The culture was then centrifuged at ∼4,500 × *g* and 4 °C for 10 min. The pellet was resuspended in 3 mL of warm LB medium supplemented with 0.7% (wt/vol) agar and 0.5 mL of the T7 lysate was added. The suspension was poured onto an LB plate and was incubated at 37 °C for 3 h. A single plaque from several emerging on the plate was purified, and the correct insertion was verified by sequencing.

T7 having *trxA* instead of the 0.4 gene and T7 having *trxA* at the end of the 0.4 gene were constructed by homologous recombination as described above, using pUC-0.4 and pUC-ctrl plasmids. The correct insertions, one in a T7 phage encoding *trxA* in place of the DNA encoding gene product (Gp) 0.4 ($T7\Delta0.4_{trxA}$) and the second in a T7 phage encoding *trxA* at the end of the 0.4 gene ($T7_{FRTtrxA}$), were verified by DNA sequencing.

Cleaving Out the *trxA* in Phages $T7\Delta0.4_{trxA}$ and $T7_{FRTtrxA}$. FLP recombinase was used to flip the *trxA* gene out from the engineered T7 phages. $T7\Delta0.4_{trxA}$ and $T7_{FRTtrxA}$ phages were diluted 1:1,000 in fresh LB. These stocks were further diluted 1:1,000 in 1 mL of a culture of *E. coli* NEB5α/pCP20, encoding the FLP recombinase (2), and aerated at 32 °C until the culture lysed. The infection cycles were repeated four times to enrich for phage with a flipped-out *trxA*. These lysates contained phages with flipped-out *trxA* that were screened as follows. Dilutions of the obtained lysates (100 μL) and diluted NEB5α/pCP20 bacteria (100 μL) were plated in LB medium supplemented with 0.7% (wt/vol) agar onto LB plates and incubated at 32 °C for 3 h. Single plaques were transferred into wells of a 96-well microtiter plate containing 50 μL LB medium. Each plaque was replicated to two fresh LB plates, with bacterial lawns of BW25113Δ*trxA* (200 μL) or BW25113Δ*ydhQ* (Table S1) (200 μL) bacteria in LB medium supplemented with 0.7% (wt/vol) agar. The plates were incubated at 37 °C for 3 h. Phages growing on the BW25113Δ*ydhQ* lawn but not on the BW25113Δ*trxA* lawn were DNA-sequenced. Phages $T7\Delta0.4_{trxA}$ and WT $T7_{FRTtrxA}$ that lost the *trxA* gene were named T7Δ0.4 and WT $T7_{FRT}$, respectively (Table S1).

Reverse-Affinity Purification Using His Tag Followed by Western Blot. Overnight culture of *E. coli* strains harboring plasmid pCA24N encoding genes *ftsZ*, *prsA*, *glpD*, *aceE*, or *hsdM* [from the ASKA library (A Complete Set of *E. coli* K-12 ORF Archive) (3)] were diluted 1:50 in LB and aerated at 30 °C to an OD_{600} of 0.2. IPTG was added to a final concentration of 1 mM, and growth was continued for an additional 2 h. At this point, each culture was infected at a MOI of ∼4 with T7 encoding either CTAP-tagged Gp0.4 or CTAP-tagged Gp1.6 as a control. After 14 min of infection, cultures were transferred directly into slurry ice. The cultures were then centrifuged at 4 °C for 20 min at 4,000 × *g*. The pellet was resuspended in lysis buffer [20 mM sodium phosphate buffer (pH 7.4), 300 mM NaCl, 10 mM imidazole, 200 μg/mL hen–egg lysozyme, 2.5 U/mL benzonase and Complete Mini EDTA-free tablet] and frozen at −80 °C overnight. The samples were thawed in a room temperature (RT) water bath and incubated for 30 min on ice. They were then frozen for 2 min in liquid nitrogen and thawed for 2.5 min in an RT water bath. This freeze–thaw cycle was repeated twice more. Cell debris was removed by centrifugation at 4 °C for 10 min at 20,000 × *g*. Supernatants were transferred into new tubes prewashed with lysis buffer, into a 50% (vol/vol) slurry of nickel nitrilo-triacetic acid (Ni NTA) beads (Thermo/Pierce), and incubated on a rotator

shaker at 4 °C for 1 h. The bead–protein complex was washed three times with wash buffer [20 mM sodium phosphate buffer (pH 7.4), 300 mM NaCl, 25 mM imidazole] and eluted with elution buffer [20 mM sodium phosphate buffer (pH 7.4), 300 mM NaCl, 250 mM imidazole]. Protein concentration was measured with a Nanodrop 2000 spectrophotometer (Thermo), and equal amounts of total protein were loaded on a 12% polyacrylamide gel. The gel was electrophoresed and subsequently stained with Imperial protein stain (Thermo/Pierce). Western blotting was performed to detect the CTAP-tagged proteins using Anti-Calmodulin-Binding Protein Epitope Tag (Merck, Upstate) according to the manufacturer's instructions.

Transduction of FtsZ. Transduction was used to replace the *ftsZ* gene with the mutant *ftsZ* gene. Bacteriophage P1 lysate was prepared as follows: the donor strain, *E. coli* encoding the *ftsZ9* allele and a kanamycin-resistance cassette in a gene located ~20 kbp from FtsZ (RK6497 strain; Table S1), was aerated overnight in LB supplemented with 25 μg/mL kanamycin at 37 °C. The probability of the kanamycin cassette to cotransduce with the *ftsZ9* allele is ~50%. This overnight culture was diluted 1:100 in LB supplemented with 25 μg/mL kanamycin, 5 mM $CaCl_2$, and 0.2% (wt/vol) glucose (Merck). After 1 h aeration at 37 °C, 0 or 100 μL of phage P1 was added. Cultures were aerated for 1–3 h, until lysis occurred. Phage P1 lysate (0 or 30 μL) was mixed with 100 μL of overnight culture of the recipient strain BW25113 (Table S1) and 1.25 μL of 1 M $CaCl_2$. After incubation at 30 °C for 30 min, 100 μL of 1 M Na-citrate (Merck) and 500 μL of LB medium were added to each tube. Infected cultures were incubated at 37 °C for 1 h, and then 3 mL of warm LB medium supplemented with 0.7% (wt/vol) agar was added to each tube. The suspension was poured onto a plate containing 25 μg/mL kanamycin. Transductants were streaked several times on LB plates containing 25 μg/mL kanamycin and their *ftsZ* gene was then DNA sequenced to differentiate between *ftsZ* and *ftsZ9* transductants.

Microscopy. The specified *E. coli* strains harboring pBAD-0.4 or pBAD18 plasmids were aerated overnight in LB medium supplemented with 100 μg/mL ampicillin at 32 °C and 0.2% glucose. These overnight cultures were diluted 1:50 in fresh LB supplemented with 100 μg/mL ampicillin at 32 °C and then induced with 0.001% L-arabinose for 0, 2, and 4 h at 32 °C with shaking. Each sample was centrifuged for 10 min, at ~4,500 × *g* and 4 °C. The bacteria were resuspended to an OD_{600} of 10. Bacteria (5 μL per slide) were viewed under an Olympus Provis AX-70 microscope. Images were taken by an Olympus DP72 camera.

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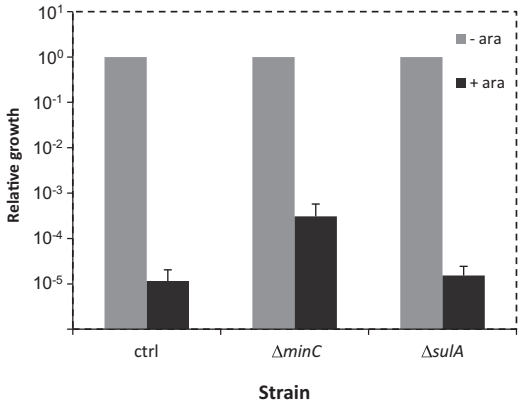

Fig. S1. Inhibition of bacterial growth by Gp0.4 in the absence of endogenous inhibitors. *E. coli* lacking the indicated genes or a control gene (*aceF*) were transformed with the plasmid encoding Gp0.4 and grown with L-arabinose induction (+ ara) or without induction (- ara). Relative growth was calculated by dividing the number of cfu obtained in the induced samples by the uninduced samples of each corresponding strain. Bars represent averages ± SD of three independent experiments.

Table S1. Bacterial strains, phages, plasmids, and oligonucleotides used in this study

Bacteria/ phages/oligonucleotides	Description	Source
Bacterial strains		
NEB5α	F$^-$, ϕ80*lacZ*ΔM15Δ*(lacZYA-argF)*, U169, *deoR*, *recA1*, *endA1*, *hsdR17* (r_k^-, m_k^+), *gal* $^-$, *phoA*, *supE44*, λ$^-$, *thi* $^-$*1*, *gyrA96*, *relA1*	New England Biolabs
BW25113Δ*trxA*	F$^-$, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ$^-$, *rph-1*, Δ*trxA::kan*, Δ*(rhaD-rhaB)568*, *hsdR514*	Ref. 1
E. coli K-12	Wild-type	Laboratory collection
BW25113	F$^-$, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ$^-$, *rph-1*, Δ(*rhaD-rhaB*)*568*, *hsdR514*	Ref. 2
BW25113Δ*aceF*	F$^-$, Δ*(araD-araB)567*, Δ*aceF::kan*, Δ*lacZ4787(::rrnB-3)*, λ$^-$, *rph-1*, Δ*(rhaD-rhaB)568*, *hsdR514*	Ref. 1
RK6497	F$^-$, Δ*(araD-araB)567*, Δ*aceF::kan*, Δ*lacZ4787(::rrnB-3)*, λ$^-$, *rph-1*, Δ*(rhaD-rhaB)568*, *hsdR514*, *ftsZ9*	This study
BW25113Δ*minC*	F$^-$, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ$^-$, *rph-1*, Δ*minC::kan*, Δ*(rhaD-rhaB)568*, *hsdR514*	Ref. 1
BW25113Δ*sulA*	F$^-$, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ$^-$, *rph-1*, Δ*sulA::kan*, Δ*(rhaD-rhaB)568*, *hsdR514*	Ref. 1
BW25113Δ*ydhQ*	F$^-$, Δ*(araD-araB)567*, Δ*lacZ4787*(::rrnB-3), λ$^-$, *rph-1*, Δ*ydhQ::kan*, Δ*(rhaD-rhaB)568*, *hsdR514*	Ref. 1
Phages		
WT T7	Wild-type T7	Laboratory collection
0.4-CTAP T7	T7 encoding Gp0.4 with CTAP tag	This study
1.6-CTAP T7	T7 encoding Gp1.6 with CTAP tag	Laboratory collection
T7Δ0.4	T7 with an FRT scar instead of 0.4 gene	This study
WT $T7_{FRT}$	T7 with an FRT scar downstream gene 0.4	This study
Plasmids		
pUC19	Cloning vector, ampicillin^r	Ref. 3
p0.4-CTAP	pUC19 cloned with CTAP and *trxA* gene, ampicillin^r	This study
pBAD18	L-arabinose-inducible expression vector, ampicillin^r	Ref. 4
pBAD-0.4	pBAD18 cloned with Gp0.4 under arabinose promoter, ampicillin^r	This study
pUC-0.4	pUC19 cloned with *trxA* gene, flanked by FRT sites and by 50 bp upstream and downstream of the DNA sequence encoding gene 0.4, ampicillin^r	This study
pUC-ctrl	pUC19 cloned with *trxA* gene, flanked by FRT sites and by 50 bp immediately downstream and upstream the stop codon of gene 0.4, ampicillin^r	This study
Oligonucleotides, 5′→3′		
RK1F	CTAGCATATGTCAAAGAACTGTACGAAAACAACAAGGCAATAGCTTTAGAATCTGCTGAGAC GCGGCCGCCAGCTGAAGC	
RK1R	CTAGTCTAGAAGTAAAGTGATAATCATAAAGGCCACTCGCTAGGAGCGACCTTGAGTCTATT ACGCCAGGTTAGCGTCGA	
RK11Fa	GACTCCCGGGGAGGAGGATGAAGAGTAATG	
RK11Ra	CTAGTCTAGATCACTCAGCAGATTCTAAAG	
RK28F	CTGTGCTCAGCGCGTGTTTC	
RK28Ra	CGCATCCAGCAGGGAGATAC	
RK28Rb	CCAACCGAAGTGTACTATAC	
RK53F	GTACCAGCTGTCAATGAATACTTGGAGGAAGTCGAGGAGTACGAGGAGGATGAAGAGTAAGA TCCGTCAGCCTGCAGTTC	
RK53R	TAAGCAGCTGAGTAAAGTGATAATCATAAAGGCCACTCGCTAGGAGCGACCTTGAGTCTATGTA GGCTGGAGCTGCTTCG	
RK60F	GTACCAGCTGAAGAACTGTACGAAAACAACAAGGCAATAGCTTTAGAATCTGCTGAGTGAGAT CCGTCAGCCTGCAGTTC	
85F	TGGTCTGATGCCTGACACCA	
SM24R1	ATTACTGCAGTCATTTGCGTAGTGCCCCTT	

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3. Lin-Chao S, Chen W-T, Wong T-T (1992) High copy number of the pUC plasmid results from a Rom/Rop-suppressible point mutation in RNA II. *Mol Microbiol* 6(22):3385–3393.
4. Guzman LM, Belin D, Carson MJ, Beckwith J (1995) Tight regulation, modulation, and high-level expression by vectors containing the arabinose PBAD promoter. *J Bacteriol* 177(14): 4121–4130.