Supporting Information

RNase I Modulates *Escherichia coli* Motility, Metabolism, and Resistance

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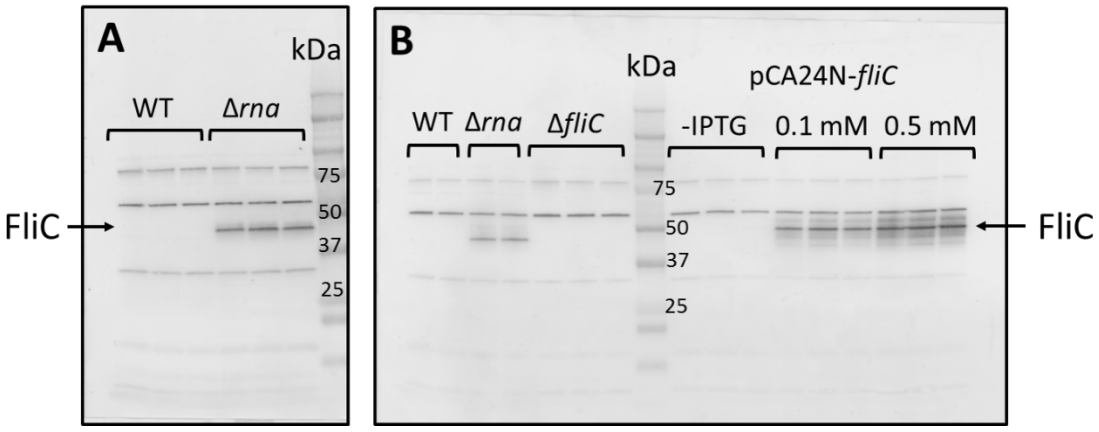
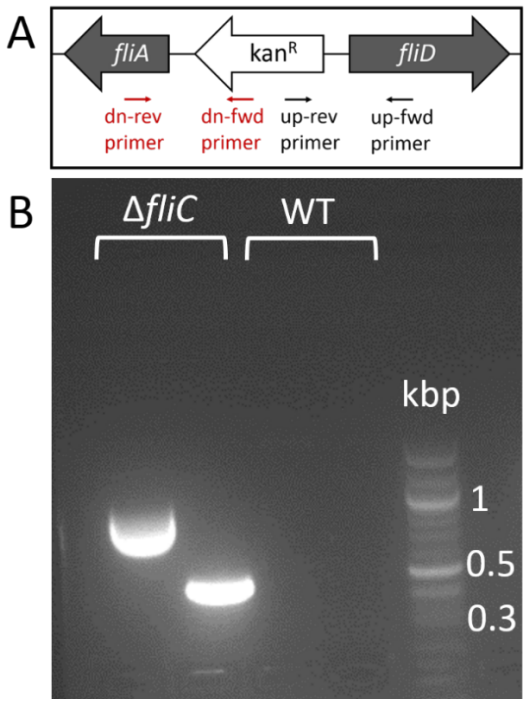
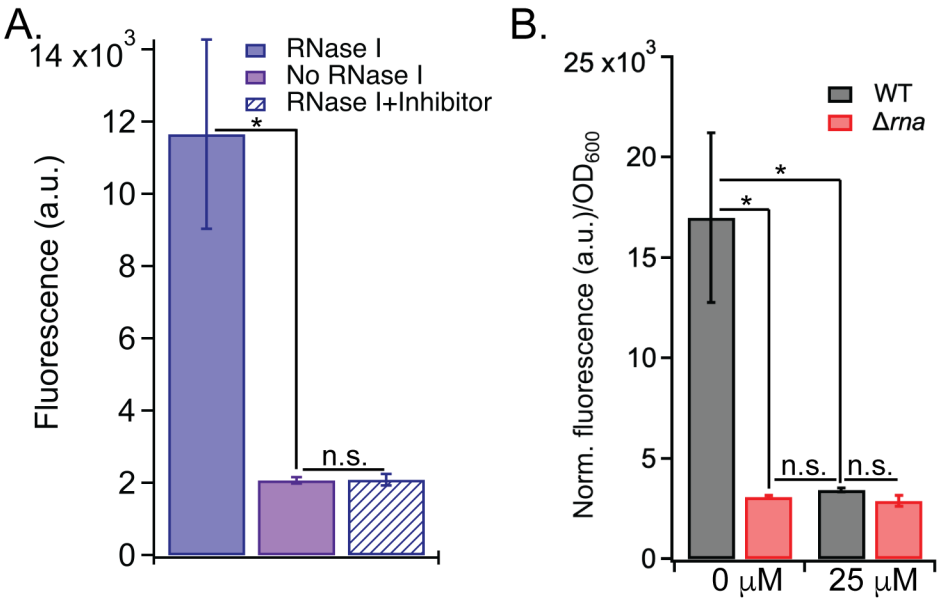


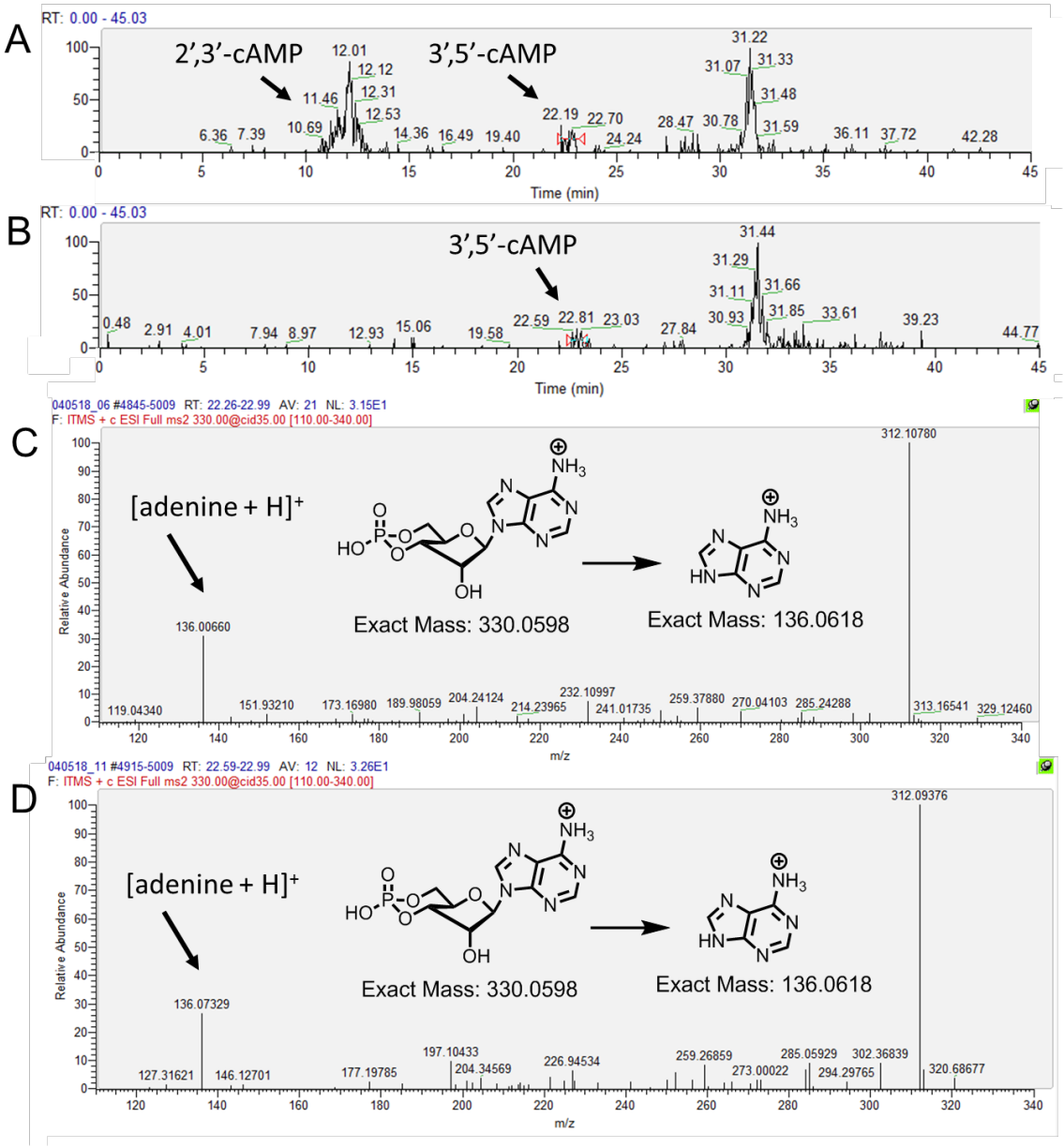
Supplementary Figure S1. (**A**) Image of western blot used for quantification of FliC expression in BW25113 WT and Δ*rna*,(see Fig. 5D in main text for quantitative data) (**B**) Western blot analysis of BW25113 *E. coli* either lacking *fliC* (Δ*fliC*) or overexpressing FliC from plasmid pCA24N-*fliC* in the presence or absence of isopropyl β-D-thiogalactopyranoside inducer (IPTG; 0.1 or 0.5 mM). These data demonstrate that the band denoted by the arrow corresponds to FliC, indicating that FliC is undetectable in WT. For all conditions assayed, each lane represents an individual biological replicate. For detailed western blot protocol, see **Materials and Methods**.



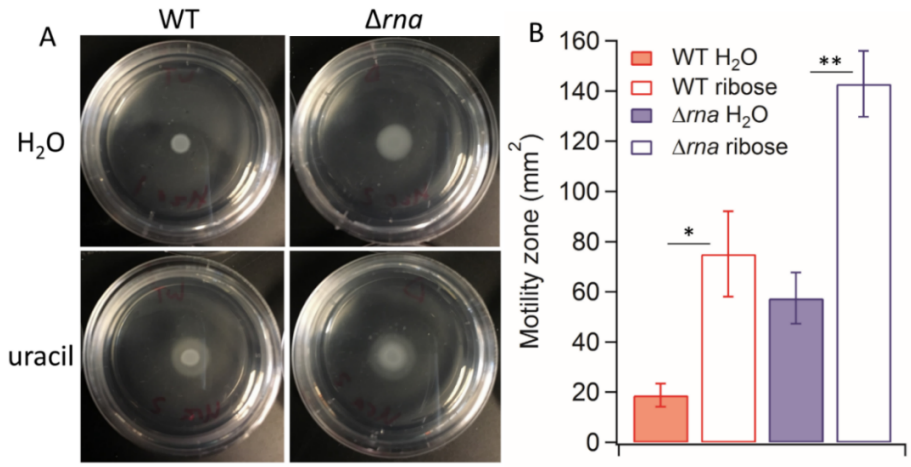
Supplementary Figure S2. PCR-based validation of *fliC*::kan^R genotype in BW25113 Δ*fliC*. (**A**) Schematic of the procedure to validate replacement of *fliC* (which lies between *fliD* and *fliA*) with the kan^R resistance cassette (not to scale). Two pairs of primers were designed to amplify across the junctions upstream and downstream of kan^R. (**B**) PCR products were analyzed on a 1.5% agarose gel, revealing products with the expected sizes in reactions containing Δ*fliC* chromosomal DNA as the template (lane 1: upstream junction; lane 2: downstream junction). In contrast, control PCR amplifications using BW25113 genomic DNA as the template produced no detectable products (last two lanes). PCR reactions were performed as described previously to validate the *rna*::kan^R genotype in BW25113 Δ*rna*.[1] For primer sequences, see **Supplementary Table S2**.



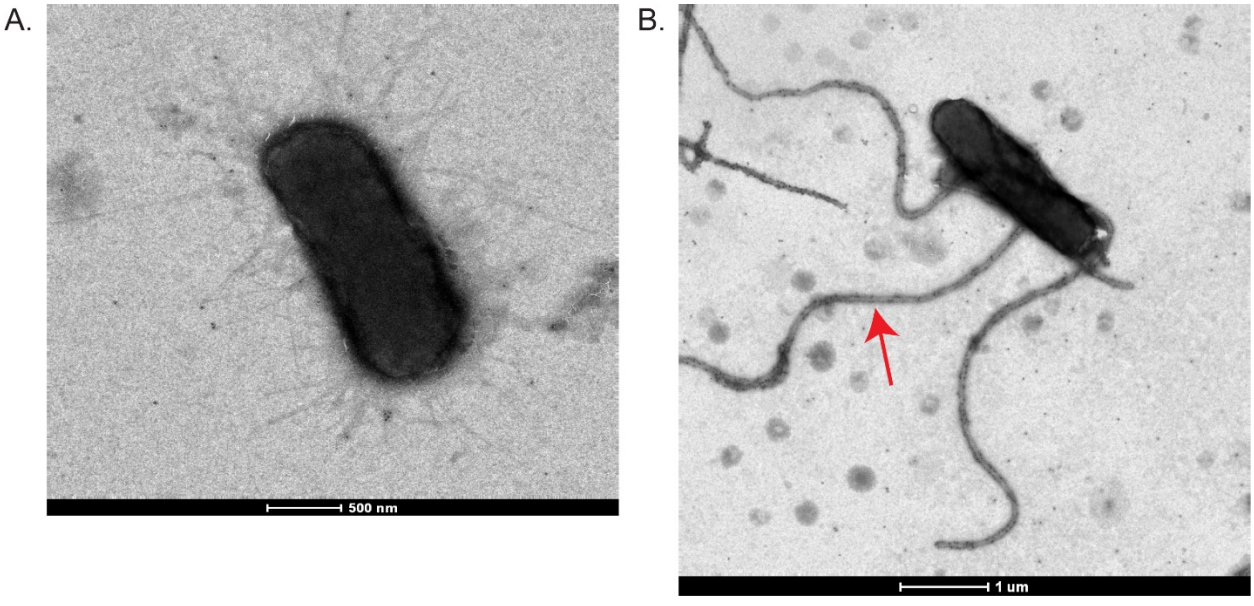
Supplementary Figure S3. Inhibition of RNase I by a phosphorothioate-modified oligonucleotide. A.) *In vitro* assessment of inhibition of purified RNase I by **Inhib1** (for sequence, see **Supplementary Table 4**). B) Inhibition of RNase I in cells by **Inhib1**, as assessed by *E. coli* WT and Δ*rna* strains. For detailed inhibition testing protocol, see **Materials and Methods** (*, $\rho < 0.05$).



Supplementary Figure S4. The intracellular concentration of adenosine 3',5'-cyclic monophosphate (3',5'-cAMP) is barely detectable in both BW25113 (WT) and the RNase I-deficient mutant (Δ*rna*). (**A, B**) LC-MS/MS chromatograms depicting the adenosine 3',5'-cyclic monophosphate (3',5'-cAMP) peak in representative extracts from BW25113 WT (**A**) and Δ*rna* (**B**). 3',5'-cAMP was detected based on the protonated parent cation with *m/z* 330.1 and quantified by the protonated adenine cation with *m/z* 136.1 (for details, see **Materials and Methods**). (**C, D**) MS/MS spectra of the 3',5'-cAMP peak (eluting at ~22 min) which depict the protonated adenine fragment ion with *m/z* 136.1 in WT (**C**) and Δ*rna* (**D**). These data demonstrate that 3',5'-cAMP is not eliciting the transcriptional changes in Δ*rna* relative to WT.



Supplementary Figure S5. WT and Δ*rna* exhibit normal chemotactic behavior in the presence of 1 mM uracil (**A**) and 1.5% w/v ribose (**B**). (**A**) The presence of the faint outer halo in the presence of uracil is indicative of a chemoattractant;[2] plate images are representative of three biological replicates. (**B**) Data represent the mean ± standard deviation of three biological replicates (* $\rho < 0.05$; ** $\rho < 0.01$). For detailed motility assay procedure, see **Materials and Methods**.



Supplementary Figure S6: Immunogold transmission electron microscopy labelling of flagellar components. (A) WT BW25113 cells lack flagellar component FliC (B) Δ*rna* cells labeled with 18 nm gold conjugated donkey anti-rabbit IgG antibody. Gold nanoparticles can be seen throughout indicating that FliC is expressed throughout the flagella.

MATERIAL AND METHODS

Bacterial strains, plasmids, general culture conditions, commercial chemicals, and statistical analyses

The *E. coli* strain BW25113 (*lacI*^{q} *rrnB*$_{T14}$ Δ*lacZ*$_{WJ16}$ *hsdR514* Δa*raBAD*$_{AH33}$ Δ*rhaBAD*$_{LD78}$)[3] and the RNase I-deficient mutant (*rna*::kan^{R}; Δ*rna*)[4] in the BW25113 strain background were obtained as stocks from the Keio collection. We previously validated disruption of the *rna* gene in Δ*rna via* locus-specific PCR amplifications.[5] For bacterial culture, discrete colonies of bacteria on Lysogeny Broth (LB)-agar plates were used to inoculate 3-mL cultures in M9 minimal medium supplemented with 0.4% glucose and 0.2% casamino acids in 15-mL plastic culture tubes, and the cultures were incubated at 37°C overnight with orbital shaking at 200-225 rpm, unless otherwise noted. The starter culture then was sub-cultured 1:100 into fresh medium and incubated under the same conditions, unless otherwise specified. Kanamycin, carbenicillin, and chloramphenicol were used at working concentrations of 25, 100, and 30 μg mL^{-1}, respectively. Analytical standards of nucleoside 5'-monophosphates and nucleoside 5'-diphosphates were purchased as sodium salts from Chem-Impex (Wood Dale, IL, USA). Adenosine 3'-monophosphate (free acid) and uridine 3'-monophosphate (disodium salt) were purchased from Sigma-Aldrich and Chem-Impex, respectively. All data depict at least n=3 biological replicates, and a two-sample t-test was employed to assess statistical significance, where equal or unequal variance was evaluated using an F-test. A P-value < 0.05 was considered statistically significant.

PCR and thermal cycling conditions for construction of plasmid pBAD33-*rna* by polymerase incomplete primer extension (PIPE) cloning

BW25113 chromosomal DNA was isolated using the GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich). The *rna* gene was amplified from the genomic template using Phusion DNA polymerase in Phusion-HF buffer (New England Biolabs). Thermal cycling was performed essentially as recommended by the manufacturer, but the final extension was omitted to enrich for incomplete extension products. An analogous thermal cycling procedure was used to amplify the pBAD33 vector. Primer sequences are provided in **Supplementary Table S1**. Following amplification, the PCR reactions were treated with *Dpn*I (New England BioLabs; 0.8 units per μL of PCR reaction) to digest the template DNA, and the products were gel-purified and extracted using the GenElute Gel Extraction Kit (Sigma-Aldrich). The vector and insert products then were mixed in a 1:3 (v/v) ratio and transformed into RbCl-competent DH5α *E. coli* for plasmid propagation. The insert sequence was confirmed by Sanger sequencing (Eurofins Genomics).

Supplementary Table S1: Primer sequences for construction of pBAD33-*rna*.

Primer ID	**Sequence (5'-3')**	**Annealing temperature (°C)**
pBAD33 vector rev	ATG GAG CCT CCT CGA ATT CGC TAG	62
pBAD33 vector fwd	CTC GAG AAG CTT GGC TGT TTT GGC	
rna insert fwd	CTA GCG AAT TCG AGG AGG CTC CAT ATG AAA GCA TTC TGG CGT AAC G	64
rna insert rev	GCC AAA ACA GCC AAG CTT CTC GAG TTA ATA ACC CGC TTT ATC AAT CAC AAA GGT	

Transcriptome profiling of WT and Δ*rna*

Analysis of mRNA transcript levels were quantified by the Emory Integrated Genomics Core and analyzed by the Emory Integrated Computational Core. Six *E. coli* pellets (three biological replicates of WT and three of Δ*rna*) were submitted for extraction and expression profiling on the Affymetrix *E. coli* Genome 2.0 Array. RNA was extracted using Qiagen miRNEasy kit (with on-column DNase treatment). Cells were lysed using 700 µL Qiazol with 100 mg acid-washed beads (150-600 µm) on the Qiagen TissueLyser at 30 Hz for 5 min. RNA was eluted in 30 µL of nuclease free water and 1 µL was used to determine the concentration on a Nanodrop 1000. An additional 1 µL was used to assess sample profiles on the Agilent 2100 using the RNA 6000 Nano assay.

Whole-Transcript Expression Analysis (Gene ST Arrays) was performed as follows. RNA (10 ng) was processed according to the GeneChip® WT Pico Reagent Kit protocol. Labeled cDNA was hybridized to the *E. coli* Genome 2.0 microarray for 16-18 hours at 45°C. Hybridized microarrays were washed and stained on an Affymetrix GeneChip Fluidics Station 450 using the appropriate chip dependent fluidics script. Intensity data were extracted using an Affymetrix 7G scanner and the Command Console software suite.

The obtained expression data from the microarray experiment were analyzed using the 'limma' package in R/Bioconductor (http://www.r-project.org). The raw data were $\log_2$ transformed and normalized across the samples by Robust Multi-array Average (RMA) normalization. The differentially expressed genes were identified on the basis of Benjamini-Hochberg (BH) multiple test adjusted *P* values (*i.e.* false-discovery rate; FDR) and fold changes (the increase in number of transcript copies). Genes with an FDR value <0.05 were considered significantly differentially expressed. Heat maps based on the z-score-normalized probe signal were created using Heatmapper (www.heatmapper.ca).[6] To visualize transcriptomic changes the PANTHER classification system (http://www.pantherdb.org)[7] was used to differentiate which enzyme classes were dysregulated by deletion of RNase I.

Gene expression data obtained from the microarray experiment have been submitted to ArrayExpress at EMBL-EBI (http://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-6095.

Motility assay procedure

Bacterial swimming motility was assayed according to a published protocol with minor modifications.[8] Motility medium (1% tryptone, 0.5% NaCl, 0.3% agar) was autoclaved and subsequently cooled in a 60°C water bath. The medium then was supplemented with antibiotics (if required) and/or ribose (1.5%) or uracil (1 mM), distributed into 60 x 15 mm petri dishes (10 mL of medium per dish), and allowed to solidify at room temperature overnight. Bacteria from an overnight culture in LB were diluted 1:100 into fresh LB and 2.5 µL were plated onto the surface of each plate. The plates were incubated at 30°C for 24 h. The area of the motility zone was quantified by measuring the zone across three diameters using a digital caliper.

Western blot quantification of FliC protein

Cultures of WT and Δ*rna* (1.5 mL) were grown in 15-mL plastic culture tubes and upon reaching OD_{600} ~0.6, 1 mL of culture was harvested by centrifugation at 10,000*g* for 5 min at RT. The pellet was flash frozen in liquid nitrogen and stored at -80°C. For western blot analysis, the cells were lysed into 100 µL of BugBuster® (EMD-Millipore) and the insoluble protein fraction was isolated according to the manufacturer's protocol. The protein was resuspended in sodium phosphate buffer (50 mM, pH 7.4), and the protein concentration of each sample was quantified in triplicate *via* Bradford assay (Bio-Rad Protein Assay Dye) to normalize protein loading for subsequent western blot analysis. Proteins were denatured by heating at 95°C for 10 min and separated by SDS-PAGE on Criterion™ TGX™ precast midi protein gels (4-20% acrylamide, Bio-Rad) at 4°C. Proteins were transferred to nitrocellulose membranes (0.2 µm, Bio-Rad) using the Mixed MW mode on the Bio-Rad Trans-Blot® Turbo™ transfer system according to the manufacturer's instructions. The blots were processed essentially as described in the Opti-4CN™ Substrate Kit (Bio-Rad). Briefly, blots were blocked by incubation in 3% Blocker solution (Bio-Rad) for 2 h at RT prior to 12 h incubation at 4°C with rabbit polyclonal anti-flagellin primary antibody (15000-fold dilution, Abcam 93713). The blots then were incubated for 1 h at RT with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (12000-fold dilution, Abcam 205718), and bands were detected by incubation with the Opti-4CN™ substrate for 15 min at RT. Blots were imaged using an Epson Perfection V600 photo scanner operating in the Professional Setting. The band intensity in the resulting 16-bit gray-scale tif image files was quantified using ImageJ (National Institutes of Health). The identity of the FliC band was confirmed by western blot analyses using strain BW25113 *fliC*::kan^R (Δ*fliC*) (obtained from the Keio collection) as a negative control and strain K12 W3110 overexpessing FliC from plasmid pCA24N-*fliC* (obtained from the ASKA collection) as a positive control. For images of all western blots, see **Supplementary Figure S1**. Disruption of the *fliC* gene with the kan^R resistance cassette in Δ*fliC* was confirmed by PCR (**Supplementary Figure S2** and **Supplementary Table S2**).

Supplementary Table S2: Primer sequences used for validation of *fliC*::kan^R genotype in BW25113 Δ*fliC*.

Primer ID	Sequence (5'-3')	Annealing temperature (°C)
Upstream junction (up-fwd)	GTA GGC GCT AAG TTT AGC GGT	64
Upstream junction (up-rev)	GTC ATA GCC GAA TAG CCT CTC CAC	
Downstream junction (dn-fwd)	TCG CAG CGC ATC GCC TTC TAT C	64
Downstream junction (dn-rev)	CAT AAC GCT GCC ACA GCG AGT	

Extraction of NDPs

Static cultures of WT and Δ*rna* (100-mL) were incubated at RT in 500-mL Ultra Yield™ flasks (Thomson Instrument Company, Oceanside, CA, USA. Upon reaching OD_{600} ~0.7-0.8, cells were harvested from 10-mL of culture by centrifugation at 2800*g* for 10 min at RT. Cells were flash frozen in liquid nitrogen and kept at -80°C until nucleotide extraction. For NDP extraction, the cell pellet was lysed by sonication on ice into 1 mL of aqueous formic acid (1 M), in analogy to published procedure.[9] The resulting lysate was freeze-dried and resuspended in 300 µL of 50 mM sodium phosphate buffer (pH 7.4) containing 0.5 µM 8-bromo adenosine 3',5'-cyclic monophosphate (8-Br 3',5'-cAMP) as internal standard (IS). The extracts were centrifuged at 12000*g* for 25 min at 4°C prior to LC-MS/MS analysis

LC-MS/MS quantification of NDPs

Nucleotides were separated and quantified by LC-MS/MS analogously to published procedure.[10] The mobile phase A consisted of 5 mM triethylammonium acetate (pH 7) in water and mobile phase B consisted of 5 mM triethylammonium acetate (pH 7) in 1:1 water/acetonitrile. The flow rate and chromatography method have been published previously.[10] Nucleotides were detected in negative-ion mode using the parent mono-anion and quantification was based on fragment ions with *m/z* 304.1, 305.1, 328.1, and 344.1 for CDP, UDP, ADP, and GDP, respectively. An internal standard (IS) method was employed for nucleotide quantification using 8-Br 3',5'-cAMP (0.5 µM) as the IS. The IS was detected in negative-ion mode using the parent mono-anion and quantified using the fragment ions with *m/z* 212.0 and 214.0 for 8-^{79}Br 3',5'-cAMP and 8-^{81}Br 3',5'-cAMP, respectively. For each nucleotide analyte a calibration curve was obtained using authentic standards spanning a concentration range from 0.02-20 µM. The calibration data then were fit to a linear regression model to calculate the concentration in the extract. To normalize for differences in bacterial culture density, nucleotide concentrations were adjusted using a value of 11.1 x 10^8 cells mL^{-1} OD_{600}^{-1} (where mL and OD_{600} are the volume and 600 nm optical density of the culture sample, respectively); this conversion factor has been determined previously for *E. coli* grown in M9 minimal medium supplemented with glucose.[11]

Adenine sensitivity assay

Cultures (2-mL) were incubated in 24-well microtiter plates (Corning Costar, sterile, untreated, polystyrene) in the presence or absence of 0.25, 0.50, or 1 mM adenine, and the OD_{600} was recorded hourly to assess the bacteriostatic effect of adenine on each strain.

Acid sensitivity assay

Acid resistance was assayed essentially as described previously.[12] Cultures of WT and Δ*rna* (3-mL) were incubated at 37 °C with shaking to OD_{600} ~0.6 and then were inoculated 1:20 into 2 mL of fresh M9 (0.4% glucose, 0.2% casamino acids) at either pH 2.5 (pH adjusted using HCl) or pH 7 (control) in a 24-well microtiter plate (VWR International, sterile, untreated, polystyrene). The cultures were incubated for 2 h and volume-normalized colony-forming units (CFU mL^{-1}) were quantified by 6x6 drop plating[13] to determine the survival rate of each strain at pH 2.5 relative to pH 7.

Carbenicillin sensitivity assay

Carbenicillin sensitivity was determined spectrophotometrically by monitoring reduction of the tetrazolium XTT as a reporter of cell viability, as described previously.[14] Cultures of WT and Δ*rna* (200 µL) were incubated in a 96-well microtiter plate (VWR International, sterile, untreated, polystyrene) in the presence of various carbenicillin concentrations spanning 0.05 to 25.6 µg mL^{-1} in two-fold serial dilutions. Additional cultures were treated with either 0 or 100 µg mL^{-1} carbenicillin as positive and negative growth controls, respectively. The plate was then incubated for 6 hours and cell viability was quantified using the XTT Cell Proliferation Kit II (Roche), essentially as described by the manufacturer. Activated XTT (50 µL) was added to each 200-µL culture and the absorbance at 450 nm was recorded on a microplate reader.

Copper Sensitivity Assay

Copper sensitivity was monitored spectrophotometrically through cell viability under both aerobic and anaerobic growth conditions. Cultures of WT and Δ*rna* were grown overnight and inoculated into fresh M9 media prior to analysis. Cultures were subjected to various concentrations of $CuSO_4$; for aerobic growth $CuSO_4$ concentrations ranged from 0 µg mL^{-1} to 800 µg mL^{-1}, while anaerobic concentrations ranged from 0 µg mL^{-1} to 3.125 µg mL^{-1}. After addition of $CuSO_4$, cultures were grown for 24 hours and growth was monitored through OD_{600}.

Quantification of Cellular Metals

The metal content in various cell pellets were quantified by the Center for Applied Isotope Studies at University of Georgia. Cells were grown in M9 medium supplemented with various metals as seen in **Supplementary Table S3**[15] under either under aerobic conditions in 50-mL Celltreat® conical tubes (sterile, polypropylene) (lids left loose for gas exchange) or under anaerobic conditions in 15-mL Hungate tubes (sterile, N51A glass). After cultures reached an OD_{600} of 0.6, 10 mL of culture were spun down at 5000g for 5 min and supernatant was

discarded. Twelve *E. coli* pellets (three biological replicates of WT and Δ*rna* grown under aerobic and anaerobic conditions) were submitted for digestion and analysis.

Supplementary Table S3: Concentration of individual metals in metal mix.

Metal	**1x media concentration**
$CaCl_2$-$2H_2O$	4 μM
$MnCl_2$-$4H_2O$	2 μM
$ZnSO_4$-$7H_2O$	2 μM
$CoCl_2$-$6H_2O$	0.4 μM
$CuCl_2$	0.4 μM
$NiCl_2$	0.4 μM
$NaMoO_4$-$2H_2O$	0.4 μM
Na_2SeO_3	0.4 μM
H_3BO_3	0.4 μM
$FeCl_3$	10 μM

RNase I Inhibition Assays

The phosphorothioate-linked RNase I inhibitors and fluorescent turn-on RNase I substrate were synthesized by Integrated DNA Technologies (for sequences, see **Table 2**). The substrate was designed with 5'-fluorescein and 3'-carboxytetramethylrhodamine (TAMRA) fluorophores to allow for fluorescence resonance energy transfer (FRET) to occur when the oligonucleotide is intact, based on previous studies on RNase A and RNase H.[16, 17]

For *in vitro* studies, all solutions were prepared using DEPC-treated nuclease-free water, and reactions were performed in 20 mM Tris-HCl (100 mM NaCl and 0.1 mM EDTA, pH 8.0) at 37°C for 1 h using 1 μM of FRET-labeled oligonucleotide substrate (optimized by varying the substrate concentration from 0.1 to 3.2 μM) and 0.04 U μL^{-1} of RNase I (1.3 nM). Substrate cleavage was monitored by recording the fluorescence of the 5'-fluorescein donor at an excitation of 485 nm and an emission of 528 nm. To confirm the absence of contaminating RNase activity, control reactions were conducted in the absence of RNase I (**Supplementary Figure S3**).

For cell-based RNase I inhibition assays, overnight cultures of WT and Δ*rna* were inoculated 1:100 in fresh M9 (0.4% glucose/0.2% casamino acids) and grown to an OD_{600} ~0.5-0.7 at 37 ˚C with shaking. From these samples, 1 mL was harvested by centrifugation at 6000*g* at room temperature for 7 min, and the pellets were washed three times with with 500 μL of phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.4, prepared with DEPC-treated water, 0.2-μM filtered, and autoclaved). After washing, the cells were resuspended in 1 mL PBS and cell density was normalized to match the least dense sample. The resulting normalized cell suspensions were pre-treated with either PBS or the indicated inhibitor (Table 3) for 30 min at RT prior to incubating with 1 μM of fluorescent substrate for 1 h at 37°C in a final volume of 20 μL. Substrate cleavage was monitored

spectrophotometrically by recording donor fluorescence, as described above for the *in vitro* assays.

Supplementary Table S4: Sequences of oligonucleotides used in RNase I inhibition studies

Identifier	Role	Sequence (5'-3')	IDT sequence code (5'-3')
sub	FRET-labeled substrate	FLdT-rA(7)-TAMdT	/5FluorT/rArA rArArA rArA/36-TAMSp/
Inhib1	inhibitor	dG*dG*dG*dG*dG	G*G*G*G*G
Inhib2	inhibitor	rU*rU*rU*rU*rU*rU	rU*rU*rU*rU*rU*rU

In the Sequence column of Supplementary Table S3 above:

FLdT denotes 2'-deoxythymidine modified with fluorescein. **rN** and **dN** denote ribonucleoside and deoxyribonucleoside residues, respectively, and **(*n*)** denotes the number of residues. **TAMdT** denotes 2'-deoxythymidine modified with TAMRA. A **dash (-)** indicates a standard phosphodiester linkage, and an **asterisk (*)** indicates a phosphorothioate linkage.

For electron microscopy studies, cells were prepared by diluting overnight cultures 1:100 in fresh M9 (0.4% glucose/0.2% casamino acids) and grown to an OD_{600} ~0.1-0.2 at 37˚ C with shaking prior to adding 100 μM or 250 μM of inhibitor. Cells were then allowed to continue growing to an OD_{600} of ~0.5-0.6 before they were prepared for SEM imaging as described below.

Electron Microscopy

For scanning electron microscopy (SEM), *E. coli* WT and Δ*rna* strains were grown to an OD ~0.5-0.6 in M9 media (0.4% glucose, 0.2% casamino acids), harvested by centrifugation at 1500*g* for 4 minutes at room temperature, and then washed with fresh M9 media. Cells were fixed by resuspending in 1 mL of 3% glutaraldehyde in PBS (pH 7.4) for 60 minutes at room temperature. Cells were then centrifuged again at 1500x*g* for 4 minutes and resuspended in fresh 3% glutaraldehyde in PBS (pH 7.4) at 4˚C until analysis. Cells were then rinsed with PBS prior to resuspending in 0.5 mL PBS. Cell suspension was applied to the center of Poly-L-Lysine coated coverslips and samples were incubated for 15 minutes at room temperature to allow cells to attach to the coverslip. Samples were dehydrated with a graded alcohol series (15, 25, 50,70, 85, 95, 100%). After dehydration, the samples were dried using a LEICA EM CPD300 critical point dryer for 2.5 hours prior to being sputter coated. SEM images were obtained using a Zeiss Sigma VP-FESEM instrument. Cell wrinkling was quantified based on a scoring metric where cells within all micrographs were scored as either wrinkled or not wrinkled based on the occurrence of a membrane deformation.

Immunogold labeling of flagella

Cells were grown to an OD_{600} of ~0.5-0.6 prior to fixation by mixing 1:1 with a solution of 4% paraformaldehyde and 0.2% glutaraldehyde fixative in PBS. Cells were then incubated with 0.1% poly-L lysine coated 300 nickel grids (formvar/carbon) by floating the grids on a drop of

cell suspension for 5 minutes. Excess liquid was wicked away and grids are incubated with a blocking solution of 1% Bovine Serum Albumin (BSA) in PBS for 15 minutes. Grids were then incubated with an Anti-Flagellin antibody (Abcam) in blocking solution for 30 minutes, followed by washing five times by floating on PBS prior to incubation with 18 nm gold conjugated donkey anti-rabbit IgG antibody (Jackson Research) in blocking solution for 30 minutes. Grids were then fixed with 2% glutaraldehyde and stained with 2% phosphotungstic acid. The grids were dried and imaged under a FEI Tecnai G2 Spirit BioTwin TEM at 120 kV.

References

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