

DksA-RNA polymerase interactions support new origin formation and DNA repair in *Escherichia coli*

Kamila K. Myka, Kira Küsters, Robert Washburn and Max E. Gottesman

SUPPLEMENTARY INFORMATION

- Supplementary experimental procedures
- Figures S1-S4
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- Supplementary references

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Confirmation of ΔrelA and ppGpp⁰ phenotypes

Strains used for viability assays were also tested on M9 minimal agar with glucose with or without 1.6 mg ml⁻¹ casamino acids or 1.6 µg ml⁻¹ serine, methionine, glycine each (Ser-Met-Gly). 10 µl of a 10-fold dilution was spread on a section of a plate and incubated at 32°C if strains contained the *dnaA46^{ts}* mutation or 37°C for all other strains. Photographs were taken after 24h incubation. $\Delta relA$ strains display impaired growth on minimal agar with Ser-Met-Gly as compared with minimal agar without amino acids. $\Delta relA \Delta spoT$ strains do not grow without casamino acids.

Phleomycin viability assay

E. coli strains were grown for 18 h at 37°C with shaking in LB broth. The cultures were then serially diluted ten-fold in M9 salts. 5 µl aliquots were spotted on LB agar plates with and without 1 µg ml⁻¹ phleomycin and the plates were incubated at 37°C. Experiments were performed at least twice; representative datasets are shown.

Figure S1

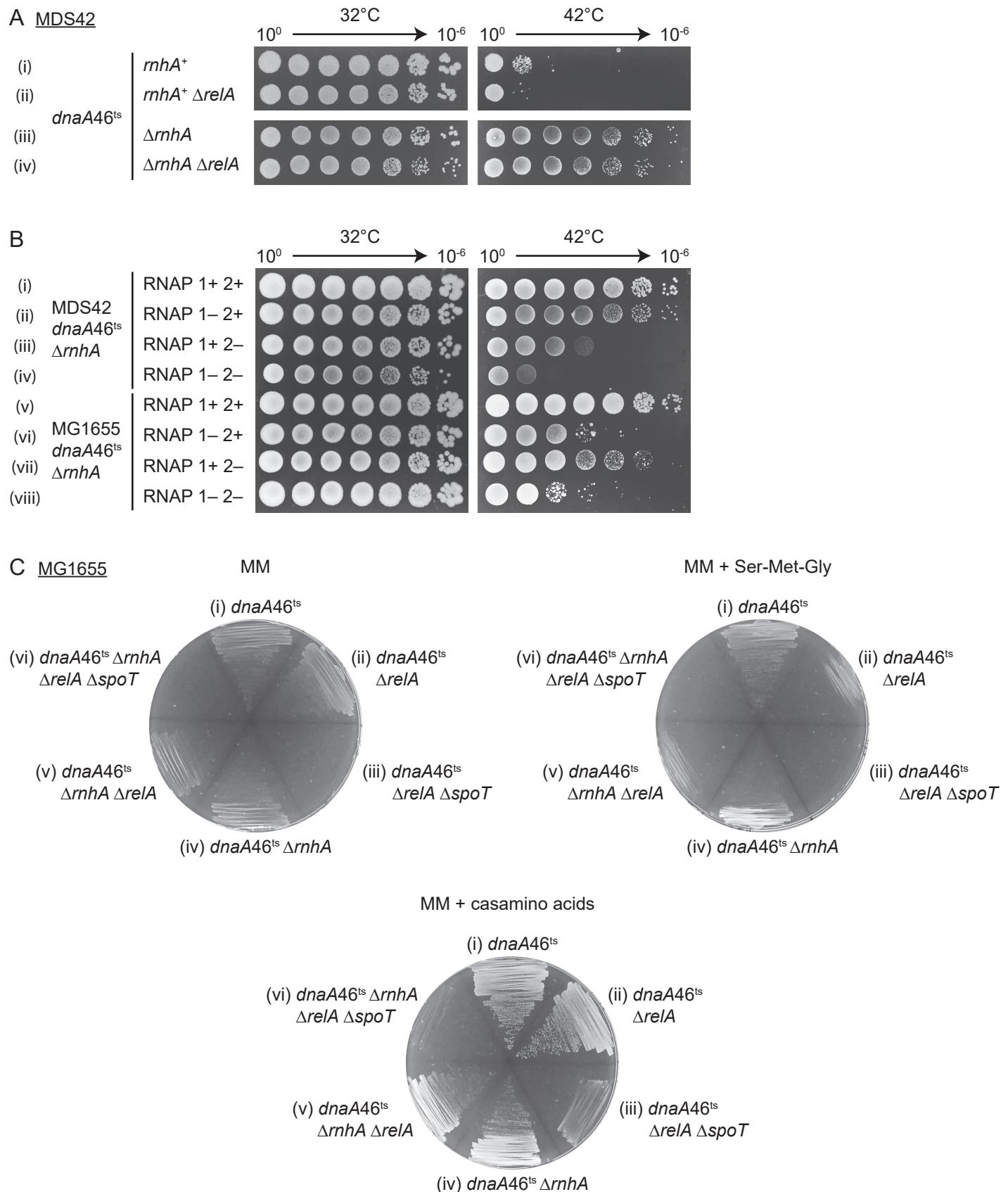


Fig. S1. The importance of (p)ppGpp for cSDR. Corresponds to Fig. 4. (A) Deletion of the gene encoding the major (p)ppGpp synthase, RelA, did not inhibit *oriC*-independent replication. Strains (i-iv): 10583, KM699, KM554, KM705. (B) RNAP (p)ppGpp binding site 2 plays a major role in cSDR in the MDS42 but not in the MG1655 background. cSDR is more impaired in RNAP (p)ppGpp binding site 1 mutants in MG1655-derived strains (i-viii): KK07B, KM901, KK09A, KM913, KM1120, KM1153, KM1165, KM1155. (C) Confirmation of *ΔrelA* and ppGpp⁰ phenotypes. Strains used are the same as in Fig. 4B (i-vi): KM712, KM1136, KM1137, KM1171, KM1173, KM1236.

Figure S2

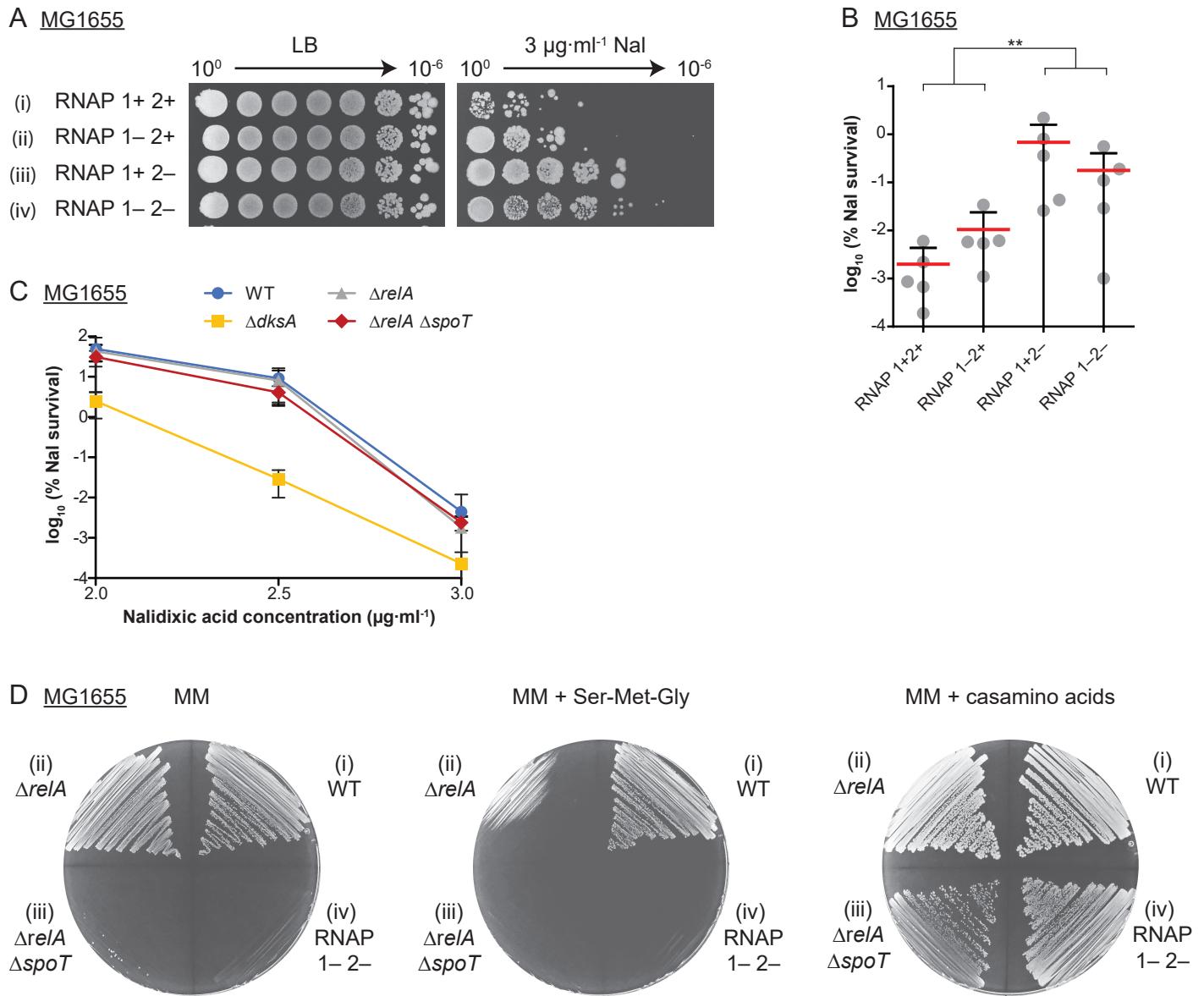


Fig. S2. RNAP (p)ppGpp-binding site 2 mutation enhances resistance to nalidixic acid. Corresponds to Fig. 5. (A) The RNAP site 1 mutation did not affect growth on Nal whereas the RNAP site 2 mutant was more resistant than wild-type. The Nal concentration used was $3 \mu\text{g ml}^{-1}$ instead of $4 \mu\text{g ml}^{-1}$ as in Fig. 5, because MG1655-derived strains are more sensitive to Nal than MDS42. Strains (i-iv): RLG14535, RLG14536, RLG14537, RLG14538. (B) Calculated percentage survival of strains on LB + Nal vs. LB alone. Graph shows mean percentage survival with one standard deviation. Statistical analysis was performed using a nonparametric two-tailed Mann-Whitney test, comparing combined data for RNAP 2+ vs. RNAP 2- mutants. ** $p < 0.01$, $n=5$. Standard deviation value higher than the mean value results in negative error bars crossing the x-axis when the y-axis is in logarithmic scale. (C) Calculated percentage survival of strains on LB + Nal vs. LB alone. Graph shows mean with standard deviation, for Nal concentration 2, 2.5, 3 $\mu\text{g ml}^{-1}$, $n=6, 6, 7$ for WT; $n=4, 4, 3$ for $\Delta dksA$ and $n=4, 4, 5$ for $\Delta relA$ and $\Delta relA \Delta spoT$. Strains as in Fig. 5C (i-iv): MG1655, KM773, RLG850, RLG847. (D) Confirmation of $\Delta relA$ and ppGpp⁰ phenotypes. Strains used are the same as in Fig. 5C (i-iii): MG1655, RLG850, RLG847. Strain (iv) is RLG14538.

Figure S3

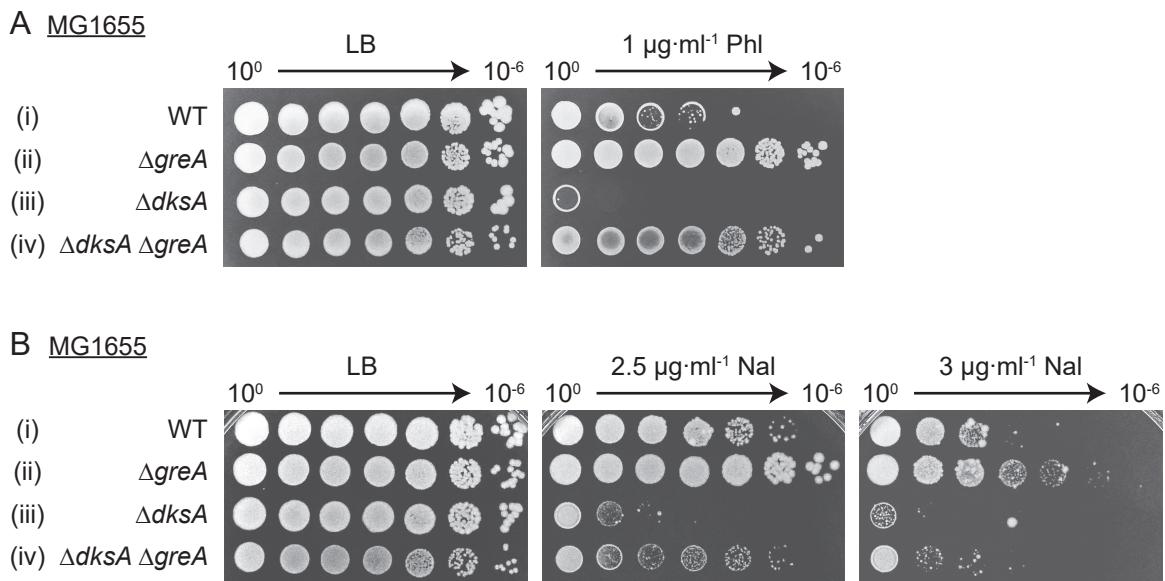


Fig. S3. Deletion of *greA* suppresses the sensitivity of a $\Delta dksA$ mutant to phleomycin (A) but not to nalidixic acid (B). Strains used are the same as in Fig. 8 (i-iv): MG1655, KM1034, KM773, KM1054.

Figure S4

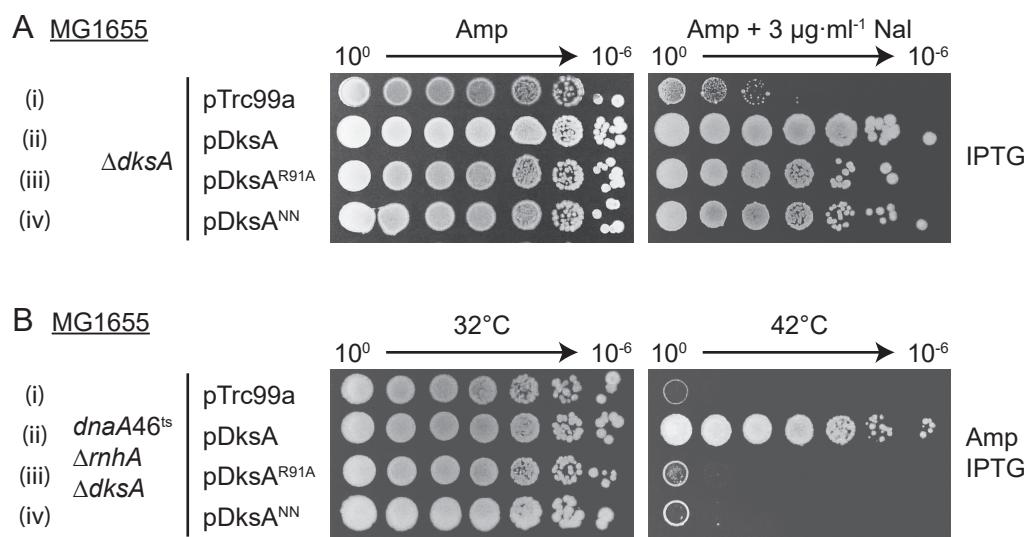


Fig. S4. DksA coiled-coil mutant proteins are able to support the repair of nalidixic acid-induced DNA damage (A) but not cSDR (B). As Fig. 11 but in the MG1655 background. Wild-type DksA protein and the DksA^{R91A} and DksA^{NN} mutants were expressed from a pTrc99a plasmids using 1 mM IPTG. Strains used: (A) KM773, (B) KM1064.

Table S1. Strains used in this study

Strain	Genotype	Construction / Source
10562	MDS42 $\Delta rnhA::cat$	MDS42 x P1.PH379
10583	MDS42 $dnaA46^{ts} tna::Tn10$	MDS42 x P1.STL8297
10598	MDS42 $rho15<amp>$	MDS42 x P1.9993
12334	MDS42 $dnaA46^{ts} tna::Tn10 \Delta greA::<kan>$	10583 x P1.JW3148
12336	MDS42 $dnaA46^{ts} tna::Tn10 \Delta greB::<kan>$	10583 x P1.JW3369
12478	MDS42 $\Delta dksA::tet rho15<amp>$	10598 x P1.11425
12481	MDS42 $\Delta dksA::<kan> rpoB^{D444G} thiC::Tn10$	KM885 x P1.JJC4490
KK04A	MDS42 $rpoZ^{WT}-kanR rpoC^{WT}-tetAR$	KK01A x P1.RLG14535
KK05A	MDS42 $rpoZ^{WT}-kanR rpoC^{N680A K681A}-tetAR$	KK01A x P1.RLG14537
KK06A	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{WT}-kanR rpoC^{WT}-tetAR$	KK02B x P1.RLG14535
KK07B	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat rpoZ^{WT}-kanR rpoC^{WT}-tetAR$	KK03A x P1.RLG14535
KK08A	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{WT}-kanR rpoC^{N680A K681A}-tetAR$	KM869 x P1.RLG14535
KK09A	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat rpoZ^{WT}-kanR rpoC^{N680A K681A}-tetAR$	KM871 x P1.RLG14535
KM554	MDS42 $dnaA46^{ts} tna::Tn10 \Delta rnhA::cat$	10583 x P1.10562
KM586	MDS42 $dnaA46^{ts} tna::Tn10 \Delta rnhA::cat \Delta greA::<kan>$	KM554 x P1.JW3148
KM588	MDS42 $dnaA46^{ts} tna::Tn10 \Delta rnhA::cat \Delta greB::<kan>$	KM554 x P1.JW3369
KM644	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s)$	
KM650	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat$	tetR removed from 10583 using chlortetacycline and fusaric acid ^a
KM712	MG1655 $dnaA46^{ts} tna::Tn10$	MG1655 x P1.10583
KM727	MG1655 $dnaA46^{ts} tna::Tn10(Tet^s)$	tetR removed from KM712 using chlortetacycline and fusaric acid ^a
KM773	MG1655 $\Delta dksA::<kan>$	MG1655 x P1.JW0141
KM777	MDS42 $\Delta rnhA::cat \Delta dksA::<kan>$	10562 x P1.JW0141
KM801	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat rpoC^{E677G} \Delta btuB3191::Tn10 \Delta yjeZ::kan$	KM650 x P1.CH2570
KM803	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta dksA::<kan>$	KM644 x P1.JW0141
KM805	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta dksA::tet$	KM644 x P1.11425
KM807	MDS42 $rpoC^{E677G} \Delta btuB3191::Tn10 \Delta yjeZ::kan$	MDS42 x P1.CH2570
KM809	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoC^{E677G} \Delta btuB3191::Tn10 \Delta yjeZ::kan$	KM644 x P1.CH2570
KM829	MDS42 $rpoC^{E677G} \Delta btuB3191::Tn10 \Delta yjeZ::kan \Delta rnhA::cat$	KM807 x P1.10562
KM882	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat \Delta dksA::<kan>$	KM650 x P1.JW0141
KM883	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat \Delta dksA::tet$	KM650 x P1.11425
KM885	MDS42 $\Delta dksA::<kan>$	MDS42 x P1.JW0141
KM899	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{\Delta 2-5}-kanR rpoC^{R362A R417A K615A}-tetAR$	KM873 x P1.RLG14538
KM901	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat rpoZ^{\Delta 2-5}-kanR rpoC^{R362A R417A K615A}-tetAR$	KM875 x P1.RLG14538
KM911	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{\Delta 2-5}-kanR rpoC^{R362A R417A K615A N680A K681A}-tetAR$	KM877 x P1.RLG14538
KM913	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat rpoZ^{\Delta 2-5}-kanR rpoC^{R362A R417A K615A N680A K681A}-tetAR$	KM879 x P1.RLG14538
KM915	MDS42 $rpoZ^{\Delta 2-5}-kanR rpoC^{R362A R417A K615A}-tetAR$	KM888 x P1.RLG14538
KM917	MDS42 $rpoZ^{\Delta 2-5}-kanR rpoC^{R362A R417A K615A N680A K681A}-tetAR$	KM890 x P1.RLG14538
KM982	MDS42 $\Delta greA::> \Delta greB::<kan>$	KM980 x P1.JW3369
KM993	MG1655 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat$	KM727 x P1.10562
KM1009	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoB^{D444G} thiC::Tn10$	KM644 x P1.12481
KM1011	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) \Delta rnhA::cat rpoB^{D444G} thiC::Tn10$	KM650 x P1.12481
KM1013	MDS42 $dnaA46^{ts} tna::Tn10(Tet^s) rpoB^{D444G} thiC::Tn10 \Delta dksA::<kan>$	KM1009 x P1.JW0141

Strain	Genotype	Construction / Source
KM1017	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat rpoB^{D444G} thiC::Tn10 ΔdksA::<kan></i>	KM1011 x P1.JW0141
KM1019	MDS42 <i>dnaA46^{ts} tna::Tn10 ΔgreA::<> ΔgreB::<kan></i>	KM982 x P1.10583
KM1021	MDS42 <i>dnaA46^{ts} tna::Tn10 ΔrnhA::cat ΔgreA::<> ΔgreB::<kan></i>	KM923 x P1.JW3369
KM1034	MG1655 <i>ΔgreA::<></i>	kanR removed from KM1027 using pCP20 ^b
KM1047	MDS42 <i>rpoB^{D444G} thiC::Tn10</i>	MDS42 x P1.12481
KM1054	MG1655 <i>ΔgreA::<> ΔdksA::<kan></i>	KM1034 x P1.JW0141
KM1058	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔgreA::<kan></i>	KM727 x P1.JW3148
KM1060	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔdksA::tet</i>	KM727 x P1.11425
KM1062	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat ΔgreA::<kan></i>	KM993 x P1.JW3148
KM1064	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat ΔdksA::tet</i>	KM993 x P1.11425
KM1066	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔgreA::<kan> ΔdksA::tet</i>	KM1058 x P1.11425
KM1068	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat ΔgreA::<kan> ΔdksA::tet</i>	KM1062 x P1.11425
KM1136	MG1655 <i>dnaA46^{ts} tna::Tn10 ΔrelA251::kan</i>	RLG850 x P1.10583
KM1137	MG1655 <i>dnaA46^{ts} tna::Tn10 ΔrelA251::kan ΔspoT207::cat</i>	RLG847 x P1.10583
KM1171	MG1655 <i>dnaA46^{ts}::Tn10 ΔrnhA::<></i>	KM716 x P1.10583
KM1173	MG1655 <i>dnaA46^{ts} tna::Tn10 ΔrnhA::<> ΔrelA251::kan</i>	KM1171 x P1.RLG847
KM1236	MG1655 <i>dnaA46^{ts} tna::Tn10 ΔrnhA::<> ΔrelA251::kan ΔspoT207::cat</i>	KM1173 x P1.RLG847
MDS42	MG1655 deleted for ~14% of the genome	(Posfai <i>et al.</i> , 2006)
MG1655	F- <i>ilvG- rfb-50 rph-1</i>	R. Lloyd laboratory
RLG847	(= CF1693) MG1655 <i>ΔrelA251::kan ΔspoT207::cat</i>	(Xiao <i>et al.</i> , 1991)
RLG850	(= CF1651) MG1655 <i>ΔrelA251::kan</i>	(Metzger <i>et al.</i> , 1989)
RSW764	MDS42 <i>ΔrnhA::cat ΔoriC::tet</i>	recombineering into RSW763

^a (Bochner *et al.*, 1980); ^b (Cherepanov and Wackernagel, 1995)

Table S2. Plasmids used in this study

Plasmid	Description	Source
pASKA	= pCA24N, P _{T5-lac} , lacI ^q , Cm ^R	(Kitagawa <i>et al.</i> , 2005)
pDksA(ASKA)	= pCA24N-JW0141 (with <i>dksA</i>)	(Kitagawa <i>et al.</i> , 2005)
pGreA(ASKA)	= pCA24N-JW3148 (with <i>greA</i>)	(Kitagawa <i>et al.</i> , 2005)
pGreB(ASKA)	= pCA24N-JW3369 (with <i>greB</i>)	(Kitagawa <i>et al.</i> , 2005)
pRLG6332	Complementation vector derived from pINIII _{AI} , P _{lp} and P _{lac} , Amp ^R	(Masui <i>et al.</i> , 1984)
pRLG6333	pRLG6332 with <i>dksA</i>	(Paul <i>et al.</i> , 2004)
pTrc99a	P _{trc} vector, lacI ^q , pUC18 EcoRI-HindIII polylinker region, Amp ^R	(Amann <i>et al.</i> , 1988)
pDksA	= pTRC-DksA-NPH, pTrc99a carrying <i>dksA</i> with N-terminal 6×His and PKA tags	(Parshin <i>et al.</i> , 2015)
pDksA ^{R91}	= pTRC-AP53, pTRC-DksA-NPH with <i>dksA</i> ^{R91A}	(Parshin <i>et al.</i> , 2015)
pDksA ^{NN}	pTRC-DksA-NPH with <i>dksA</i> ^{D71N D74N}	S. Borukhov laboratory

Table S3. Strains used in supplementary figures

Strain	Genotype	Construction / Source
KM699	MDS42 <i>dnaA46^{ts} tna::Tn10 ΔrelA251::kan</i>	10583 x P1.RLG850
KM705	MDS42 <i>dnaA46^{ts} tna::Tn10 ΔrnhA::cat ΔrelA251::kan</i>	KM554 x P1.RLG850
KM1120	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat rpoZ^{WT}-kanR rpoC^{WT}-tetAR</i>	KM1116 x P1.RLG14535
KM1153	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat rpoZ^{Δ2-5}-kanR rpoC^{R362A R417A K615A-tetAR}</i>	KM1149 x P1.RSW863
KM1155	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) ΔrnhA::cat rpoZ^{Δ2-5}-kanR rpoC^{R362A R417A K615A N680A K681A-tetAR}</i>	KM1151 x P1.RSW863

Strain	Genotype	Construction / Source
KM1165	MG1655 <i>dnaA46^{ts}</i> <i>tna::Tn10(Tet^s) Δrnha::cat rpoZ^{WT}-kanR rpoC^{N680A K681A}-tetAR</i>	KM1126 x P1.RSW863
RLG14535	MG1655 <i>rpoZ^{WT}-kanR rpoC^{WT}-tetAR (1+2+)</i>	(Ross <i>et al.</i> , 2016)
RLG14536	MG1655 <i>rpoZ^{Δ2-5}-kanR rpoC^{R362A R417A K615A}-tetAR (1-2+)</i>	(Ross <i>et al.</i> , 2016)
RLG14537	MG1655 <i>rpoZ^{WT}-kanR rpoC^{N680A K681A}-tetAR (1+2-)</i>	(Ross <i>et al.</i> , 2016)
RLG14538	MG1655 <i>rpoZ^{Δ2-5}-kanR rpoC^{R362A R417A K615A N680A K681A}-tetAR (1-2-)</i>	(Ross <i>et al.</i> , 2016)

Table S4. Strains used for construction

Strain	Genotype	Construction / Source
9993	HME57 <i>rho15<amp></i>	D. Court laboratory
11105	MDS42 <i>ΔgreA::<kan></i>	MDS42 x P1.JW3148
11425	MDS42 <i>dksA::tet</i>	MDS42 x P1.RLG7238
CH2570	<i>lacZ-U118 ΔyjaZ::kan ΔbtuB3191::Tn10 rpoC^{E677G}</i>	(Satory <i>et al.</i> , 2013)
JJC4490	JJC40 <i>rpoB^{D444G} thiC::Tn10</i>	(Baharoglu <i>et al.</i> , 2010)
JW0141	F- $\Delta(araD-araB)567 \Delta dksA761::kan \Delta lacZ4787(:rrnB-3) λ^- rph-1 \Delta(rhaD-rhaB)568 hsdR514$	(Baba <i>et al.</i> , 2006)
JW0204	F- $\Delta(araD-araB)567 \Delta rnhA733::kan \Delta lacZ4787(:rrnB-3) λ^- rph-1 \Delta(rhaD-rhaB)568 hsdR514$	(Baba <i>et al.</i> , 2006)
JW3148	F- $\Delta(araD-araB)567 \Delta lacZ4787(:rrnB-3) λ^- ΔgreA788::kan rph-1 \Delta(rhaD-rhaB)568 hsdR514$	(Baba <i>et al.</i> , 2006)
JW3369	F- $\Delta(araD-araB)567 \Delta lacZ4787(:rrnB-3) λ^- ΔgreB740::kan rph-1 \Delta(rhaD-rhaB)568 hsdR514$	(Baba <i>et al.</i> , 2006)
KK01A	MDS42 <i>rpoZ^{WT}-kanR</i>	MDS42 x P1.RLG14535
KK02B	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{WT}-kanR</i>	KM644 x P1.RLG14535
KK03A	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) Δrnha::cat rpoZ^{WT}-kanR</i>	KM650 x P1.RLG14535
KM716	MG1655 $\Delta rnha::<>$	kanR removed from RSW899 using pCP20 ^b
KM869	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoC^{N680A K681A}-tetAR</i>	KM644 x P1.RLG14537
KM871	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) Δrnha::cat rpoC^{N680A K681A}-tetAR</i>	KM650 x P1.RLG14537
KM873	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoC^{R362A R417A K615A}-tetAR</i>	KM644 x P1.RLG14536
KM875	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) Δrnha::cat rpoC^{R362A R417A K615A}-tetAR</i>	KM650 x P1.RLG14536
KM877	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoC^{R362A R417A K615A N680A K681A}-tetAR</i>	KM644 x P1.RLG14538
KM879	MDS42 <i>dnaA46^{ts} tna::Tn10(Tet^s) Δrnha::cat rpoC^{R362A R417A K615A N680A K681A}-tetAR</i>	KM650 x P1.RLG14538
KM888	MDS42 <i>rpoC^{R362A R417A K615A}-tetAR</i>	MDS42 x P1.RLG14536
KM890	MDS42 <i>rpoC^{R362A R417A K615A N680A K681A}-tetAR</i>	MDS42 x P1.RLG14538
KM923	MDS42 <i>dnaA46^{ts} tna::Tn10 Δrnha::cat ΔgreA::<></i>	kanR removed from KM586 using pCP20 ^b
KM980	MDS42 <i>ΔgreA::<></i>	kanR removed from 11105 using pCP20 ^b
KM1027	MG1655 <i>ΔgreA::<kan></i>	MG1655 x P1.JW3148
KM1114	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{WT}-kanR</i>	KM727 x P1.RLG14535
KM1116	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) Δrnha::cat rpoZ^{WT}-kanR</i>	KM993 x P1.RLG14535
KM1122	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{Δ2-5}-kanR</i>	KM727 x P1.RLG14536
KM1126	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{WT}-kanR rpoC^{N680A K681A}-tetAR</i>	KM1114 x P1.RLG14537
KM1149	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{Δ2-5}-kanR rpoC^{R362A R417A K615A}-tetAR</i>	KM1122 x P1.RLG14536
KM1151	MG1655 <i>dnaA46^{ts} tna::Tn10(Tet^s) rpoZ^{Δ2-5}-kanR rpoC^{R362A R417A K615A N680A K681A}-tetAR</i>	KM1122 x P1.RLG14538
PH379	RFM430 <i>rnhA::cat</i>	(Usongo <i>et al.</i> , 2008)
RLG7238	RLG5950 <i>dksA::tet</i>	(Rutherford <i>et al.</i> , 2007)
RSW763	MDS42 <i>Δrnha::cat pSIM6</i>	MDS42 pSIM6 x P1.PH379
RSW863	MG1655 <i>Δrnha::cat</i>	MG1655 x P1.PH379

Strain	Genotype	Construction / Source
RSW899	MG1655 $\Delta rnhA::kan$	MG1655 x P1.JW0204
STL8297	$dnaA46^{\text{ts}}$ $tta::Tn10$	(Foti <i>et al.</i> , 2005)

^b (Cherepanov and Wackernagel, 1995)

SUPPLEMENTARY REFERENCES

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