

Supplemental information

Table S1 Strains and plasmids used in this study.

Strains or plasmids	Description	Sources
Strains		
<i>E. coli</i> DH5 α	F- 80 <i>lacZ</i> M15 (<i>lacZYA</i> – <i>argF</i>)U169 <i>eoR</i> <i>recA1endA1 hsdR17 phoA supE44-thi-1</i> <i>gyrA96 relA1</i>	Laboratory stock
<i>E. coli</i> KA413	llv <i>thyA tyrA</i> (Am) <i>trpE9829</i> (Am) <i>metE</i> <i>deo supF6</i> (Ts) <i>dnaA46</i>	Gift from T. Katayama
<i>E. coli</i> BL21	F- <i>ompT gal dcm lon hsdSB</i> (rB- mB-) λ (DE3 [<i>lacI lacUV5</i> -T7 gene 1 <i>ind1 sam7 nin5</i>])	Laboratory stock
BL21 derivatives		
<i>his-dnaA</i>	[BL21] <i>his-dnaA</i> (cm ^R)	Laboratory stock
Δ <i>cobB-his-dnaA</i>	[BL21] Δ <i>cobB-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
Δ <i>yfiQ-his-dnaA</i>	[BL21] Δ <i>yfiQ-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
Δ <i>ackA-his-dnaA</i>	[BL21] Δ <i>ackA-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
Δ <i>pta-his-dnaA</i>	[BL21] Δ <i>pta-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
Δ <i>ackAcobB-his-dnaA</i>	[BL21] Δ <i>ackAcobB-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
<i>E. coli</i> BW25113	<i>LacIq rrnBT14</i> Δ <i>lacZWJ16hsdR514</i> Δ (<i>araBAD</i>)AH33 Δ (<i>rhaBAD</i>)LD78	Laboratory stock Laboratory stock
<i>BW25113</i> derivatives		

<i>his-dnaA</i>	[BW25113] <i>his-dnaA</i> (cm ^R)	Laboratory stock
Δ <i>cobB-his-dnaA</i>	[BW25113] Δ <i>cobB-his-dnaA</i> (cm ^R)	Laboratory stock
Δ <i>yfiQ-his-dnaA</i>	[BW25113] Δ <i>yfiQ-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
Δ <i>ackA-his-dnaA</i>	[BW25113] Δ <i>ackA-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock
Δ <i>pta-his-dnaA</i>	[BW25113] Δ <i>pta-his-dnaA</i> (cm ^R &Kan ^R)	Laboratory stock

Plasmids

pCDSS	Spe ^R , used for plasmid complementation assay	Gift from Peter Schultz
pCDSS-K	pCDSSara harboring wild-type <i>dnaA</i>	Laboratory stock
pCDSS-Q	pCDSSara harboring <i>dnaA</i> (K243Q)	This work
pCDSS-R	pCDSSara harboring <i>dnaA</i> (K243R)	This work
pAcKRS-3	Kan ^R , coding for <i>M. barkeri</i> pyrrolysine tRNA synthetase	Gift from J Chin
pCDF-PyIT	Spe ^R , derivated from pCDFduet and harboring the <i>pyIT</i> gene	Gift from J Chin
pCDF-PyIT- <i>dnaA</i>	Spe ^R , used for protein expression	Laboratory stock
pCDF-PyIT- <i>dnaA</i> (K243TAG)	Spe ^R , used for site-specific acetylated DnaA expression	This work
pBKS- <i>oriC</i>	Amp ^R , derivated from pBluescript II KS(+) and containing the 469-bp <i>oriC</i> fragment	Laboratory stock

Table S2 Primers used in this study.

Primer Name	Primer Sequence
K243Q F	CAGTTTTTTTGCTAATCAAGAACGATCTCAGGAA
K243Q R	TTCCTGAGATCGTTCTTGATTAGCAAAAAACTG
K243R F	CAGTTTTTTTGCTAATCGAGAACGATCTCAGGAA
K243R R	TTCCTGAGATCGTTCTCGATTAGCAAAAAACTG
K243Ac F	CAGTTTTTTTGCTAATTAGGAACGATCTCAGGAA
K243Ac R	TTCCTGAGATCGTTCCTAATTAGCAAAAAACTG
pET22b- <i>dnaA</i> F	TCTCCATTAGGTGTCACITTCGCTTTGG
pET22b- <i>dnaA</i> R	TCTCCTCGAGCGATGACAATGTTCTGAT
pBKS- <i>oriC</i> F	CCGGAGCTCGCCAATGATGATGACGTCAA
pBKS- <i>oriC</i> R	GGGGTACCAATCGGTATTGGTAGTCGTG
pCDF- <i>dnaA</i> F	CCGCCATGGGCCATCATCATCATCATCATTCACTTTGCTT TGGCAGCA
pCDF- <i>dnaA</i> R	GGCTCGAGTTACGATGACAATGTTCTGATT
5'FAM- <i>oriC</i> F	5'FAM-CCCGGGCCGTGGATTCTACT
5'FAM- <i>oriC</i> R	5'FAM-CTCGAGGCAGAACTCAAAGA
5'FAM-P <i>dnaA</i> F	TGGTCATTAAATTTTCCAATATG
5'FAM-P <i>dnaA</i> R	GGCGGACTCCACTCGAAC
5'FAM-DARS1 F	5'FAM-GGGGATAGGGGCTGGAGACA
5'FAM-DARS1 R	5'FAM-CGTGCAAGCCGCGTATTC

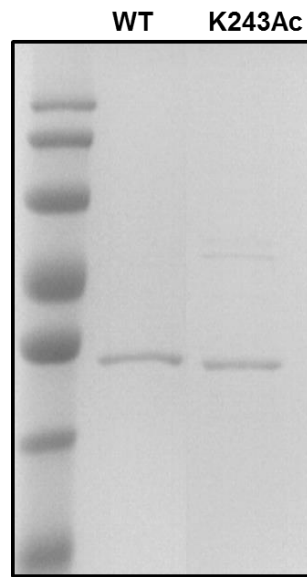


Figure S1. The purified wild-type and DnaA K243Ac proteins. The wild-type and DnaA K243Ac (200 ng) were resolved on 10% SDS-PAGE and stained with Coomassie blue.

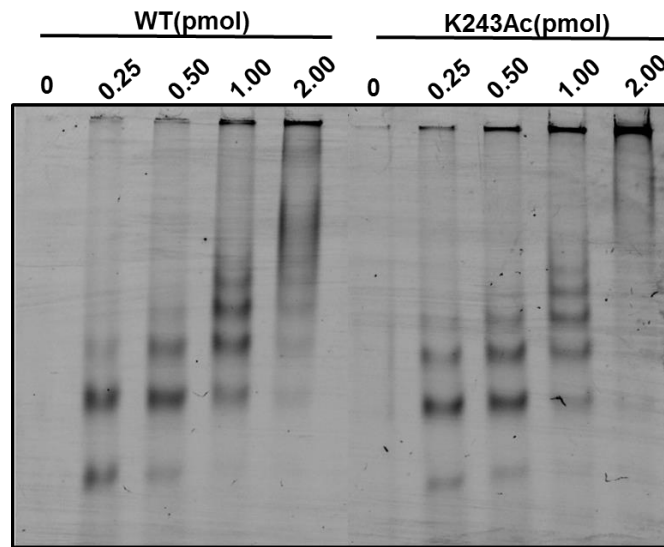


Figure S2. Binding activities of DnaA to DARS1. Various amounts (0-2 pmol) of the wild-type and K243Ac ADP-DnaA proteins were incubated with FAM-labeled DARS1 for 5 min at 30°C. Reaction products were analyzed by 5% PAGE and detected by FUJIFILM FLA7000.

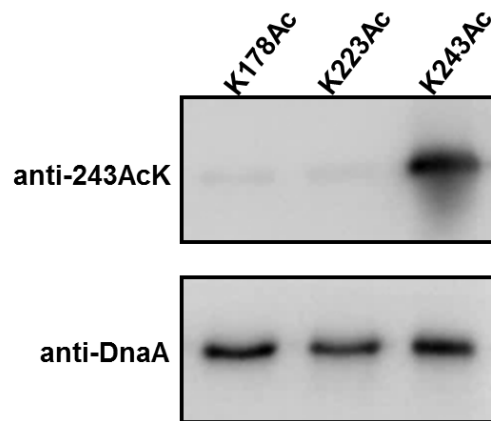


Figure S3. Evaluation of anti-K243Ac antibody by Western blot. Three different site-specific acetylated DnaA were purified, and probed by anti-DnaA antibody and anti-K243Ac antibody, respectively. Western blots were independently repeated at least three times.

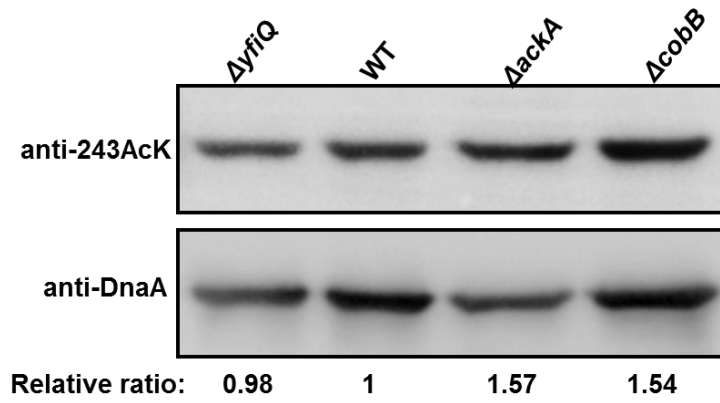


Figure S4. *In vivo* acetylation level of DnaA K243 in different BW25113 strains. Native DnaA protein was purified from the wild-type *E. coli* strain BW25113 and three mutant strains at the mid-exponential phase, and they were probed by anti-DnaA antibody and anti-K243Ac antibody, respectively. Western blots were independently repeated at least three times.