PNAS
www.pnas.org

Supplementary information for: DksA-DnaJ redox interactions provide a signal for the activation of bacterial RNA polymerase

Ju-Sim Kim, Lin Liu, Liam F. Fitzsimmons, Yang Wang, Matthew A. Crawford, Mauricio Mastrogiovanni, Madia Trujillo, James K. Till, Rafael Radi, Shaodong Dai, and Andrés Vázquez-Torres

Corresponding authors:
andres.vazquez-torres@ucdenver.edu
rradi@fmed.edu.uy

This PDF includes:

Supplementary Materials and Methods.

Remetallation assays. Zinc-deficient DksA was prepared by treating DksA with 1 mM methyl methanethiosulfonate (MMTS), in the presence of 0.1 mM diethylenetriaminepentaacetic acid (DTPA), for 30 minutes at room temperature in a Bactron I anaerobic chamber. After incubation, an aliquot from the reaction was set aside to confirm zinc chelation. Excess MMTS and zinc chelates were removed from the remaining reaction volume by double exchange into degassed 50 mM potassium phosphate buffer, pH 7.0 using Zeba Spin desalting columns. Zinc-deficient, MMTS-modified DksA was subsequently reduced by treatment with 10 mM (final) DTT for 1 h at room temperature under an anaerobic environment. DTT was then removed by double exchange into degassed 50 mM potassium phosphate buffer, pH 7.0. Following exchange, an aliquot of reduced, zinc-deficient DksA was removed from the anaerobic chamber to measure the protein concentration using the Pierce 660 reagent. The protein concentration of zinc-containing DnaJ was determined in parallel. In the anaerobic chamber, reduced, zinc-deficient DksA and zinc-containing DnaJ were combined at a 1:1 molar ratio, and this mixture was incubated for 1 h at 37°C.

Replicate aliquots (25 μl) from the prepared samples (reduced, zinc-containing DksA; reduced, zinc-deficient DksA; reduced, zinc-deficient DksA + zinc-containing DnaJ; and zinc-containing DnaJ) were removed from the anaerobic chamber and treated with $ONOO^-$ (570 μM, final), prepared in ice-cold 10 mM NaOH, for 5 minutes at 37°C or urea (5.7 M) for 15 minutes at 95°C. Zinc release was subsequently quantified by spectrometry (OD_{500}) using 150 μM (final) of the metallochromic indicator 4-(2-pyridylazo) resorcinol (PAR). Zinc concentrations were calculated from standard curves prepared using $ZnCl_2$ standards in the presence of $ONOO^-$ or urea, as appropriate. $ONOO^-$ was synthesized with H_2O_2 and acidified nitrite (1)

Growth in minimal medium. Strains grown overnight in LB at 37°C were diluted 1:100 in EG minimal media (0.2% $MgSO_4$, 2% $C_6H_8O_7{\cdot}H2O$, 10% K_2HPO_4, 3.5% $Na(NH_4)HPO_4{\cdot}4H_2O$, and 4% D-glucose, pH 5.5) and incubated at 37°C. Cell growth was determined by following OD_{600} over time.

Heat shock response. Strains grown overnight in LB broth at 37°C were sub-cultured 1:100 in LB broth. The specimens were incubated at 37°C to 45°C. Bacterial growth was determined by recording cfu over time.

Detection of DksA and DnaJ in immunoblots. Wild-type and Δ*dnaJ Salmonella* expressing the *dksA::*FLAG were grown in LB broth at 37°C overnight, harvested, washed in PBS buffer, and resuspended in PBS buffer at an OD_{600} of 0.3. Where indicated, the bacterial cells were treated with 100 μM H_2O_2 at 37°C. After 30 min of incubation with shaking, the bacteria were harvested for Western blot analysis. In addition, stationary phase *Salmonella* subcultured 1:100 in LB broth were grown to an OD_{600} of 0.5-0.7. Cells were disrupted by an ultrasonication liquid process, and protein concentrations were determined by BCA (Thermo Fisher Scientific). Samples were separated on 12% SDS-PAGE gels, and the proteins were transferred onto nitrocellulose membranes by electro-transfer. Blots were probed with anti-FLAG monoclonal (Sigma-Aldrich, Burlington, MA) or anti-DnaJ polyclonal (Enzo, Farmingdale, NY) antibodies, followed by HRP-conjugated anti-mouse IgG or anti-rabbit IgG antibodies (GE Healthcare), respectively. Immunoblots were developed as recommended using the ECL prime Western blotting detection system (GE Healthcare), and imaged with a ChemiDoc XRS imaging system (Bio-Rad, Hercules, CA). As a control, DnaK was detected with anti-DnaK monoclonal antibodies (MBL International, Woburn, MA) followed by HRP-conjugated anti-mouse IgG (GE Healthcare).

Measurement of (p)ppGpp. Bacterial strains were grown to OD_{600} of approximately 0.2 (1.5 doubling times) in MOPS minimal media containing glucose, all amino acids, 0.4 mM K_2HPO_4, and 10 μCi/mL of [^{32}P]-labeled orthophosphate. Cells were treated with 10-25 μM H_2O_2 or 70 μg/ml tetracycline before 0.4 ml of ice-cold 50% formic acid was added to the cultures. Extracts were incubated on ice for at least 20 min and the specimens were centrifuged at 13,000 rpm for 5 min. Ten μl of ice-cold extracts were spotted along the bottom of polyethyleneimine-cellulose thin layer chromatography (TLC) plates (20 x 20 cm; EDM Millipore, Billerica, MA). Plates were air-dried and the nucleotides were separated for 1 h with a 1.25 M K_2HPO_4 solvent system, pH 3.4, in a TLC chamber. TLC autoradiograms were visualized with phosphor screens and a phosphorimager (Bio-Rad, Hercules, CA). Relative nucleotide levels were quantified with the ImageJ software (NIH, Rockville, MD).

Table S1. DksA partner molecules identified by mass spectrometric analysis.

Protein	Description
RpoC	RNA polymerase β’
RpoB	RNA polymerase β’
NusA	Modifies transcription through interactions with RNA polymerase; impacts elongation, readthrough, termination, and anti-termination
Tig	Trigger factor, promotes folding of newly synthesized proteins
DnaJ	Chaperone protein
RpoA	RNA polymerase α
RfaD	ADP-L-glycero-D-mannoheptose-6-epimerase
SegA	negative modulator of the initiation of chromosome replication
StpA	15 kDa of DNA binding protein
HupA	10 kDa of DNA-binding protein HU-α

Table S2. Zinc and thiol content of purified DnaJ protein variants in 8 M urea.

Protein	Zinc release (μM)	Thiol content (μM)
WT DnaJ	10.55 ± 0.71	49.27 ± 0.78
DnaJ C186H	11.02 ± 0.35	43.52 ± 0.13
DnaJ C268A	10.30 ± 0.15	43.80 ± 0.77

*5 μM of the indicated proteins were used to measure zinc and thiol content.

Table S3. Bacterial strains used in this study.

Strain	Relevant characteristics	Reference
Salmonella		
14028s	wild-type of *S. Typhimurium*	ATCC
AV17142	Δ*dksA*::FRT (pWSK29::TAP)	lab strain
AV10342	Δ*dksA*::FRT (pWSK29::*dksA*::TAP)	lab strain
AV17180	Δ*dksA*::FRT, *rpoC::*6His::Cm	This study
AV08016	*dksA*::3xflag::FRT	lab strain
AV15188	*dksA*::3xflag::FRT, Δ*dnaJ*::Km	This study
AV17134	*dksA*::1xflag::Cm	This study
AV17143	*dksA* K98A::1xflag::Cm	This study
AV15172	Δ*dnaJ*::Km	This study
AV15184	Δ*dnaJ*::Km (pWSK29::*dnaJ*)	This study
AV16068	Δ*dnaJ*::Km (pWSK29::*dnaJ* C186H)	This study
AV17055	Δ*dnaJ*::Km (pWSK29::*dnaJ* C268A)	This study
AV0663	Δ*relA*::FRT	(2)
AV14005	Δ*relA::FRT* Δ*spoT*::FRT	(3)
E. coli		
DH5α	*supE*44 Δ*lacU*169 (ϕ80 *lacZ* ΔM15) *hsdR*17 *recA*1 *endA*1 *gyrA*96 *thi*-1 *relA*1	(4)
BTH101	F-, *cya*-99, *ara*D139, *gal*E15, *gal*K16, *rps*L1 (Str^r), *hsd*R2, *mcr*A1, *mcr*B1	Euromedex
BL21(DE3)	*fhuA2 [lon] ompT gal (λ DE3) [dcm]* Δ*hsdS* *λ DE3 = λ sBamHIo* Δ*EcoRI-B* *int::(lacI::PlacUV5::T7 gene1 i21* Δ*nin5*	Invitrogen
Origami B(DE3) pLysS	*E. coli* K12 F- *ompT hsdSB*(rB- mB-) *gal dcm lacY*1 *aphC* (DE3) *gor*522::Tn10 *trxB pLysS* (Cm^r, Km^r, Tet^r)	Novagen
AV15092	HTH101 (pKT25 ::*dksA*, pUT18C)	This study
AV15093	HTH101 (pKT25 ::*dksA*, pUT18C ::*stpA*)	This study
AV15094	HTH101 (pKT25 ::*dksA*, pUT18C ::*hupA*)	This study
AV15095	HTH101 (pKT25 ::*dksA*, pUT18C ::*tig*)	This study
AV15096	HTH101 (pKT25 ::*dksA*, pUT18C ::*dnaJ*)	This study
AV15099	HTH101 (pKT25 ::*rpoA*, pUT18C)	This study
AV15100	HTH101 (pKT25 ::*rpoA*, pUT18C ::*stpA*)	This study
AV15101	HTH101 (pKT25 ::*rpoA*, pUT18C ::*hupA*)	This study
AV15102	HTH101 (pKT25 ::*rpoA*, pUT18C ::*tig*)	This study
AV15103	HTH101 (pKT25 ::*rpoA*, pUT18C ::*dnaJ*)	This study

170	AV10267	BL21(DE3) (pGEX6p-DksA)	(5)
171	AV10266	BL21(DE3) (pGEX6p-DksA C114S)	(5)
172	AV10258	BL21(DE3) (pGEX6p-DksA C135S)	(5)
173	AV17115	BL21(DE3) (pGEX6p-DksA all C to S)	(5)
174	AV18004	BL21(DE3) (pGEX6p-DksA 146)	This study
175	AV18005	BL21(DE3) (pGEX6p-DksA 141)	This study
176	AV18006	BL21(DE3) (pGEX6p-DksA 136)	This study
177	AV18007	BL21(DE3) (pGEX6p-DksA 131)	This study
178	AV17165	BL21(DE3) (pGEX6p-DksA K98A)	This study
179	AV17014	BL21(DE3) (pET22b-DnaJ)	This study
180	AV17138	BL21(DE3) (pET22b-Tig)	This study
181	AV17015	Origami B(DE3) pLysS (pET22b-DnaJ-I)	This study
182	AV17018	Origami B(DE3) pLysS (pET22b-DnaJ-II)	This study
183	AV17019	Origami B(DE3) pLysS (pET22b-DnaJ-III)	This study
184	AV17200	Origami B(DE3) pLysS (pET22b-DnaJ-IV)	This study
185	AV17203	Origami B(DE3) pLysS (pET22b-DnaJ-V)	This study
186	AV17204	Origami B(DE3) pLysS (pET22b-DnaJ-VI)	This study
187	AV17139	BL21(DE3) (pET22b-DnaJ C186H)	This study
188	AV18117	BL21(DE3) (pET22b-DnaJ C167H)	This study
189	AV17140	BL21(DE3) (pET22b-DnaJ C268A)	This study

190

191

Table S4. Plasmids used in this study.

Plasmid	Relevant characteristics	Reference
pET-22b(+)	*ori* pBR322, C-terminal His·Taq fusion vector, Pn^r	Novagen
pET22b::DnaJ	pET-22b(+) + 1.1-kb DNA containing *dnaJ*, Pn^r	This study
pET22b::DnaJ-I	pET-22b(+) + 0.37-kb DNA containing truncated *dnaJ*, Pn^r	This study
pET22b::DnaJ-II	pET-22b(+) + 0.59-kb DNA containing truncated *dnaJ*, Pn^r	This study
pET22b::DnaJ-III	pET-22b(+) + 0.64-kb DNA containing truncated *dnaJ*, Pn^r	This study
pET22b::DnaJ-IV	pET-22b(+) + 0.52-kb DNA containing truncated *dnaJ*, Pn^r	This study
pET22b::DnaJ-V	pET-22b(+) + 0.68-kb DNA containing truncated *dnaJ*, Pn^r	This study
pET22b::DnaJ-VI	pET-22b(+) + 0.79-kb DNA containing truncated *dnaJ*, Pn^r	This study
pET22b::DnaJ C167H	pET-22b(+) + 1.1-kb DNA containing *dnaJ* C167H, Pn^r	This study
pET22b::DnaJ C186H	pET-22b(+) + 1.1-kb DNA containing *dnaJ* C186H, Pn^r	This study
pET22b::DnaJ C268A	pET-22b(+) + 1.1-kb DNA containing *dnaJ* C268A, Pn^r	This study
pET22b::Tig	pET-22b(+) + 1.30-kb DNA containing *tig*, Pn^r	This study
pET22b::RpoC	pET-22b(+) + 4.23-kb DNA containing *rpoC*, Pn^r	This study
pGEX6p	GST fusion expression vector, Pn^r	(6)
pGEX6::*dksA*	pGEX6p + 454-bp DNA containing *dksA*, Pn^r	(5)
pGEX6::*dksA* C114S	pGEX6p + 0.45-kb DNA containing *dksA* C114S, Pn^r	(5)
pGEX6::*dksA* C135S	pGEX6p + 0.45-kb DNA containing *dksA* C135S, Pn^r	(5)
pGEX6::*dksA* ΔC	pGEX6p + 0.45-kb DNA containing *dksA* C114S C117S C135S C138S, Pn^r	This study
pGEX6::*dksA* 146	pGEX6p + 439-bp DNA containing the C-terminal truncated *dksA* (Δ5 amino acid), Pn^r	This study
pGEX6::*dksA* 141	pGEX6p + 424-bp DNA containing the C-terminal truncated *dksA* (Δ10 amino acid), Pn^r	This study
pGEX6::*dksA* 136	pGEX6p + 409-bp DNA containing the C-terminal truncated *dksA* (Δ15 amino acid), Pn^r	This study
pGEX6::*dksA* 131	pGEX6p + 409-bp DNA containing the C-terminal truncated *dksA* (Δ20 amino acid), Pn^r	This study
pGEX6::*dksA* K98A	pGEX6p + 0.45-kb DNA containing *dksA* K98A, Pn^r	This study
pKD3	template vector for FRT-flanked Cm^r cassette, Cm^r Pn^r	(7)
pKD13	template vector for FRT-flanked Km^r cassette, Km^r Pn^r	(7)
pKT25	pSU40 derivative with T25 domain of CyaA, MCS at the end of T25, Kan^r	Euromedex
pKT25::*dksA*	pKT25 plasmid with *cyaAT25-dksA* fusion, Kan^r	This study
pKT25::*rpoA*	pKT25 plasmid with *cyaAT25-rpoA* fusion, Kan^r	This study
pBluescriptSK(+)	Standard cloning vector 2958 bp, Pn^r	Stratagene
pSK::*dksA*::1xflag::Cm	pSK(+) + 1.4-kb DNA containing *dksA*::flag,Cm^r Pn^r	This study
pSK::*dksA*K98A::1xflag::Cm	pSK(+) + 1.4-kb DNA containing *dksAK98A*::flag, Cm^r Pn^r	This study

pSK::*rpoC*'::6His::Cm	pBluescriptKS(+) + 1.89-kb DNA containing 539 bp C-terminal *rpoC* fused with 6His + 1,035 bp Cm^r cassette + 318 bp *rpoC* flanking region to construct *rpoC*::6His::Cm, Cm^r and Pn^r	This study
pTIM	bla *rrnB* & *rpoC* term pBluescript	(8)
pTIM-*livJ*	pTim + 1.34-kb DNA containing P*livJ* (-240) and *livJ*	This study
pTIM-*rpsM*	pTim + 0.56-kb DNA containing P*rpsM* (-203) and *rpsM*	This study
pUT18C	pUC19 derivative with T18 domain of CyaA, MCS at the 3' start of T18, Pn^r	Euromedex
pUT18C::*dnaJ*	pUT18 plasmid with *dnaJ-cyaAT18* fusion, Pn^r	This study
pUT18C::*hupA*	pUT18 plasmid with *hupA-cyaAT18* fusion, Pn^r	This study
pUT18C::*stpA*	pUT18 plasmid with *stpA-cyaAT18* fusion, Pn^r	This study
pUT18C::*tig*	pUT18 plasmid with *tig-cyaAT18* fusion, Pn^r	This study
pWSK29	low copy plasmid, *lacZ*α, Pn^r	(9)
pWSK29::TAP	pWSK29 + 0.42-kb DNA containing TAP, Pn^r	(10)
pWSK29::*dksA*::TAP	pWSK29 + 1.07-kb DNA containing p*dksA::dksA*::TAP, Pn^r	This study
pWSK29::*dnaJ*	pWSK29 + 1.49-kb DNA containing p*dnaK::dnaJ*, Pn^r	This study
pWSK29::*dnaJ* C186H	pWSK29 + 1.49-kb DNA containing p*dnaK::dnaJ* C186H, Pn^r	This study
pWSK29::*dnaJ* C268A	pWSK29 + 1.49-kb DNA containing p*dnaK::dnaJ* C268A, Pn^r	This study

Table S5. Oligonucleotides used in this study.

Strain	Primer Sequence (5' → 3')
Δ*dnaJ*::Km	F: CCGCCCGTGTATGCATGTTAAGGGCAGATAAAAAGAGATGGTG TAGGCTGGAGCTGCTTC
	R : TACACCCGGGCTGAAGAAAAATACAACGGGAAAAGATTAATTC CGATTCCGGGGATCCGTCGACC

Plasmid	
pET22b::DnaJ	F : CATATGGCGAAAAGAGATTACTACGAG
	R : CTCGAGAGTCAAATCGTCAAAGAATTTTTTC
pET22b::DnaJ-I	F : CATATGGCGAAAAGAGATTACTACGAG
	R : CTCGAGCAAATCAGCCCCACGCGCCGC
pET22b::DnaJ-II	F : CATATGGCGAAAAGAGATTACTACGAG
	R : CTCGAGCAGCGTACCGCGTCCCTGACA
pET22b::DnaJ-III	F : CATATGGCGAAAAGAGATTACTACGAG
	R : CTCGAGTTCAACACGCCCATGACCGTG
pET22b::DnaJ-IV	F : CATATGGAAAAGAGTAAAACTCTGTCC
	R : CTCGAGAGTCAAATCGTCAAAGAATTTTTTC
pET22b::DnaJ-V	F : CATATGGCTGGCACGCAACCGCAAACC
	R : CTCGAGAGTCAAATCGTCAAAGAATTTTTTC
pET22b::DnaJ-VI	F : CATATGTTGCGTTATAACATGGATC
	R : CTCGAGAGTCAAATCGTCAAAGAATTTTTTC
pET22b::RpoC	F : CATATGAAAGATTTATTAAAGTTTCTG
	R : CTCGAGCTCGTTATCAGAACCGCCC
pET22b::Tig	F : CATATGCAAGTTTCAGTTGAAACCACTCAG
	R : AAGCTTCGCCTGCTGGTTCATCAGCTC
pKT25::*dksA*	F : TCTAGAGCAAGAAGGGCAAAACCGTAAAAC
	R : GGATCCCCCGCCATCTGTTTTTCGCG
pKT25::*rpoA*	F : GGATCCTCGTCAGCGATGCTTGCCGG:
	R : GGATCCTCGTTATCAGAACCGCCCAGAC
pSK::*rpoC*'::6His::Cm	
1. *rpoC*'::6His DNA	F: ATTTGAATTCGTTAAGATTAACGATAAACACATCGAAG
	R: ATCGTCTAGATTAATCAGTGGTGGTGGTGGTGGTGCTCGAGCT
2. Cm cassette	F : ACTAGTCATGGTCCATATGAATATCC
	R : ACTAGTGTGTAGGCTGGAGCTGCTTC
3. *rpoC* flanking	F : ATCGACTAGTTCGTTAAATGGTGGAGGGGTATTT
	R : ATCGAAGCTTGCTTTTTTATCCGCGCTGG
pSK::*dksA*::1xflag::Cm	F : ATCGTAGAATTCATGCAAGAAGGGCAAAACCGTAAAACATC
	R :CCGCGGATCCTTACTTGTCGTCATCGTCTTTGTAGTCACCCGC CATCTGTTTTTCGCG
pTim-*livJ*	F : GGAATTCCAATACGTTTGCCCGATGG
	R : ACTCTGCAGTCACTTAGCGTCTGTCGC
pTim-*rpsM*	F : ATCGAATTCCAATACGTTTGCCC
	R : ACTAAGCTTTCACTTAGCGTCTGTCGC
pUT18C::*dnaJ*	F : TCTAGAGAAAAGAGATTACTACGAGA
	R: GAGCTCCGAGTCAAATCGTCAAAGAA
pUT18C::*hupA*	F: TCTAGACAAGACTCAACTGATTGATG
	R: GAGCTCTTAACTGCGTCTTTCAGAGC
pUT18C::*stpA*	F: TCTAGATTTGATGTTACAGAACTTAAATAATATCC

	R: GAGCTCATTAAGAAATCATCCAGAGATTTCC
pUT18C::*tig*	F: TCTAGAAGTTTCAGTTGAAACCACTCAG
	R: GAGCTCGCCTGCTGGTTCATCAGCTC
pWSK29::TAP	F:AAGAATTCATGAAGCGACGATGGAAAAAGAATTTCATAGC
	R:AACTGCAGTTATTCTTTGTTGAATTTGTTATCCGCTTTCGGT
pWSK29::*dksA*::TAP	F : ATACTCGAGCGAACCAGTACCCATAAC
	R : ATCGAATTCACCCGCCATCTGTTTTTCG
pWSK29::*dnaJ*	
1. *pdnaK* DNA fragment	F : CCGCTCGAGGCAGGCCGACGGAAATCGTTAACAC
	R : GAATTCCTAAACGTCTCCACTAAAAATTCGGTCATCAT
2. *dnaJ* DNA fragment	F : GAATTCATGGCGAAAAGAGATTACTACGAGATTTTAGGCGT
	R : CGCGGATCCTTAGCGAGTCAAATCGTCAAAGAATTTTTTCACG

Point mutations	
dksA K98A	F : CCGTGAGCGCAAACTGATCAAA*GCG*ATCGAGAAGACGCTG
	R : CAGCGTCTTCTCGAT*CGC*TTTGATCAGTTTGCGCTCACGG
dnaJ C167H	F : CCTGACCAGAACCATG*ATG*GGTCGGACAGGTTTGCG
	R : CGCAAACCTGTCCGACC*CAT*CATGGTTCTGGTCAGG
dnaJ C186H	F : CCCTGACAGTGTGG*GTG*GGTCTGCTGTACAGC
	R : GCTGTACAGCAGACC*CAC*CCACACTGTCAGGG
dnaJ C268A	F : CAATAATCTTTAT*GCA*GAAGTGCCGATCAACTTTG
	R : GATCGGCACTTC*TGC*ATAAAGATTATTGCC

Truncated DksA proteins	
dksA 146	F : ATCGGATCCATGCAAGAAGGGCAAAACCGTAAAAC
	R : ATCGAATTC**TTA**TTCGCGAATTTCAGCCAGCGTTTTG
dksA 141	F : ATCGGATCCATGCAAGAAGGGCAAAACCGTAAAAC
	R : ATCGAATTC**TTA**CAGCGTTTTGCAGTCGATGCA
dksA 136	F : ATCGGATCCATGCAAGAAGGGCAAAACCGTAAAAC
	R : ATCGAATTC**TTA**GATGCACAGATCGGCTGTTGG
dksA 131	F : ATCGGATCCATGCAAGAAGGGCAAAACCGTAAAAC
	R : ATCGAATTC**TTA**TGTTGGACGCGCTTCCAG

Real time qRT-PCR	
rpoD	F: GTGGCTTGCAATTCCTTGAT
	R: AGCATCTGGCGAGAAATA
	Probe: 6-FAM-ATAAGTTCGAATACCGTCGCG-3BHQ-1
livJ	F: CGCAGGGCTGAAAACCCA
	R: CACACGAATGCGCCGCTA
	Probe: 6-FAM-TCAGCGGAAGGCTTACTGGTC-3BHQ-1
hisG	F : CAGGCCGTTTAAGCGATGATTCACGAG
	R : AATACCGAGATCGACCACGCCATCC
	Probe: 6-FAM-ATCGGCATGTTTTCCGCCATCGCAATCAGG-3BHQ-1
***In vitro* transcription**	
livJ	F : GGAATTCCAATACGTTTGCCCGATGG
	R : TGCACTGCAGTGCATATTTCACCGCGACGAGC
hisG	F : TAAGGCGTAAAAGTGGTTTAG
	R: CACGCGCAGGATATCAATCGGC

* Restriction enzyme sites are underlined.

** Point mutation sites are indicated in italics.

*** Stop codons are marked in bold.

Supplementary figure legends

Fig. S1. Recombinant DnaJ and DksA proteins and biochemical pull-down assay. Protein-protein interactions were evaluated in (A) a bacterial two-hybrid system in which T25 and T18 fragments of adenylate cyclase are fused to bait and prey proteins, respectively, and by (B) biochemical pull-down assays using recombinant proteins in the absence of DTT. GST and GST-DksA proteins were used as bait, whereas DnaJ and Tig were used as prey. DnaJ in the pull-downs assays was detected by Western blotting. (C, D, E) Input proteins used in the biochemical pull-down assays corresponding to Fig. S1B, 1C and 1D as shown by Coomassie-stained SDS-PAGE gels.

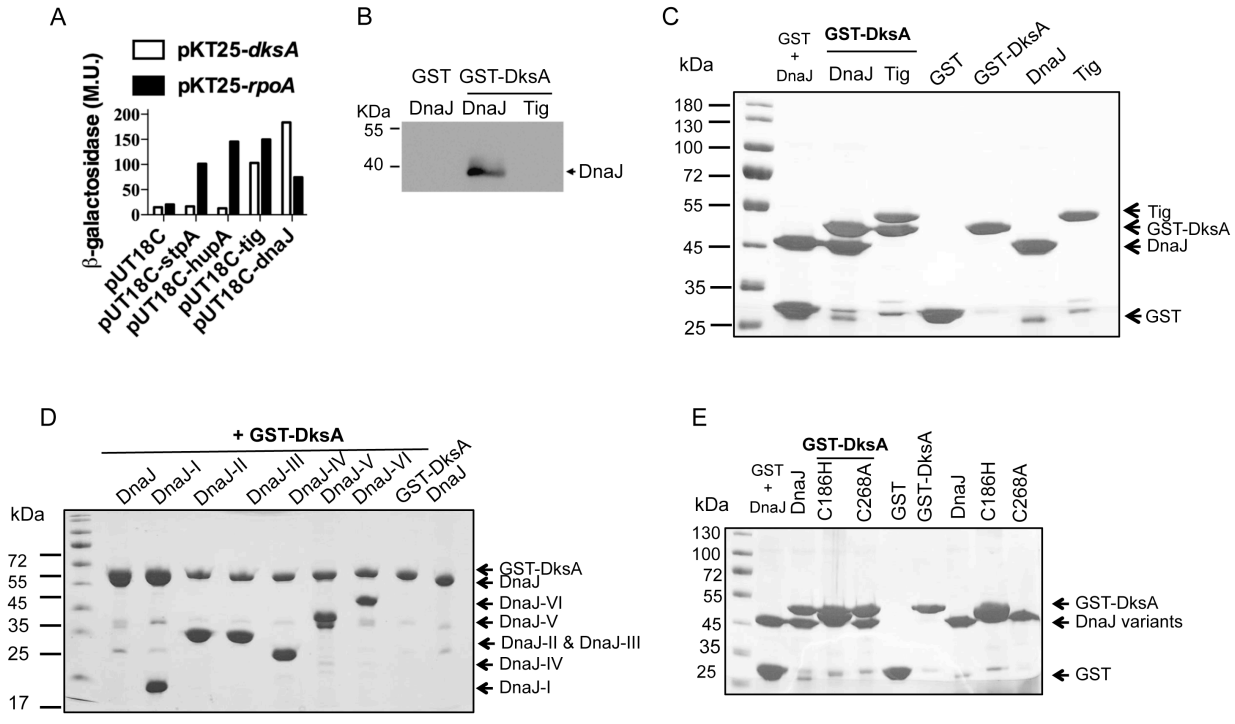


Fig. S2. Zinc content of recombinant DksA variants. (A-C) Input proteins (5 μg) used for the pull-down assays shown in Fig. 2A, D and E were visualized in Coomassie-stained SDS-PAGE gels. (D) Purified DksA proteins used for CD analysis shown in **Fig. 2G**. (E) Determination of zinc content from 25 μM of recombinant, WT DksA and truncated variants after treatment with 50-1000 μM H_2O_2 as measured spectrophotometrically with the zinc chelator PAR using regression analysis of standard curves prepared with $ZnCl_2$.

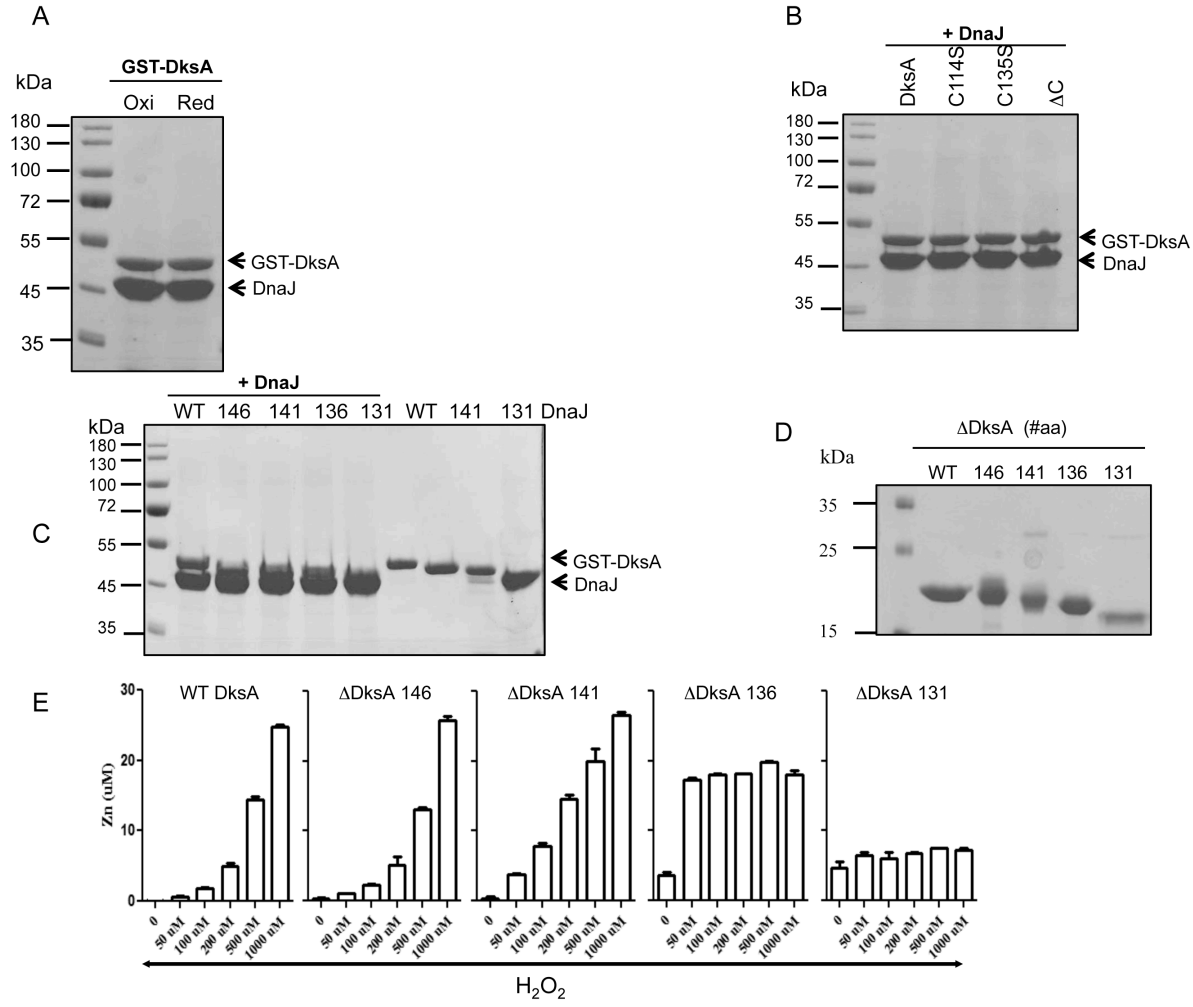


Fig. S3. Characterization of recombinant DnaJ and determination of DksA remetallation and redox state in aerobic cultures. (A) Thiol content and zinc release after treatment of 3.33 μM DnaJ recombinant protein with increasing concentrations of H_2O_2 or $ONOO^-$. Thiol content, as calculated from the molar extinction coefficient of TNB, was measured spectrophotometrically by following the reaction of sulfhydryl groups with DTNB. The amount of thiol detected in untreated samples corresponds to about 2 moles per mole of protein, likely reflecting modification of the two cysteines in the C-terminal domain. Addition of up to 10 molar excess of H_2O_2 or $ONOO^-$ did not change the estimated thiol content. Neither H_2O_2 nor $ONOO^-$ released zinc from DnaJ, as determined using PAR. Together these findings suggest that that cysteine residues in DnaJ site 1 and site 2 zinc fingers may be resistant to oxidants. (B) Thiol content and zinc release after 3.33 μM of recombinant DnaJ were treated with increasing concentrations of urea. (C) Proteins used in the MS analysis done in **Fig. 3B** were visualized in SDS-PAGE gels stained with Coomassie Brilliant Blue. (D) Zinc release from: 1. zinc-containing DnaJ (DnaJ alone); 2. reduced, zinc-deficient DksA pre-incubated for 1 h at 37°C with an equimolar amount zinc-containing DnaJ ($zinc^{neg}$ DksA + DnaJ); 3. reduced, zinc-deficient DksA ($zinc^{neg}$ DksA) prepared by DTT reduction and desalting of DksA pre-treated with the sulfhydryl-reactive compound MMTS in the presence of the chelating agent DTPA; 4. reduced, zinc-containing DksA ($zinc^{pos}$ DksA). Zinc release was measured by spectrometry using the metallochromic indicator PAR following exposure of the samples to $ONOO^-$ (570 μM final) or urea (5.7 M final). Zinc concentrations were calculated from standard curves prepared using $ZnCl_2$ standards in the presence of $ONOO^-$ or urea, as appropriate. Oxidation by $ONOO^-$ elicits zinc release from DksA, but not DnaJ. Urea elicits zinc release from both DksA and DnaJ. Upon exposure to $ONOO^-$, minimal zinc release was observed from the $zinc^{neg}$ DksA + DnaJ sample, indicating that DnaJ did not remetallate zinc-deficient DksA under the experimental conditions examined. (E) Redox state of thiol groups in DksA cysteine residues in aerobic, log phase *Salmonella* was evaluated by Western blot analysis after derivatization with the alkylation

agent AMS. Immunoblot analysis was performed to detect DksA-FLAG proteins.

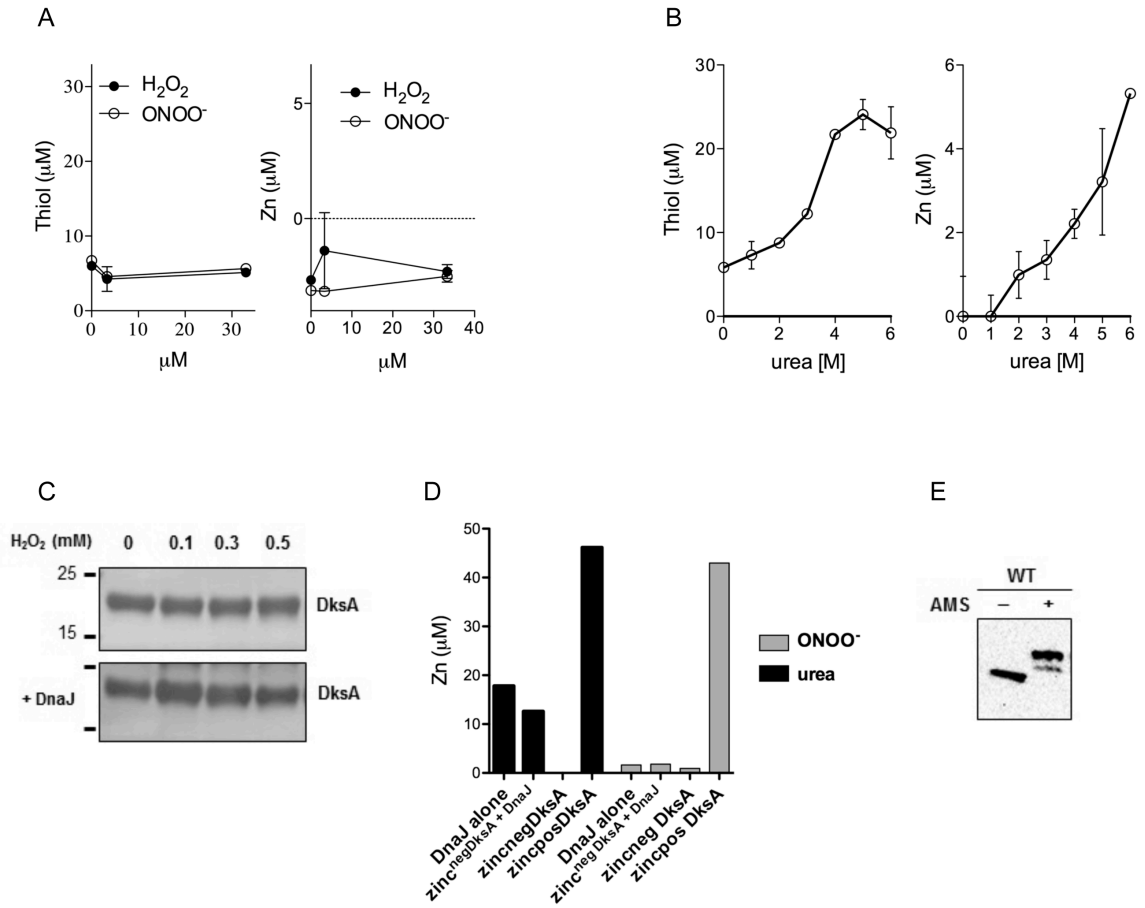
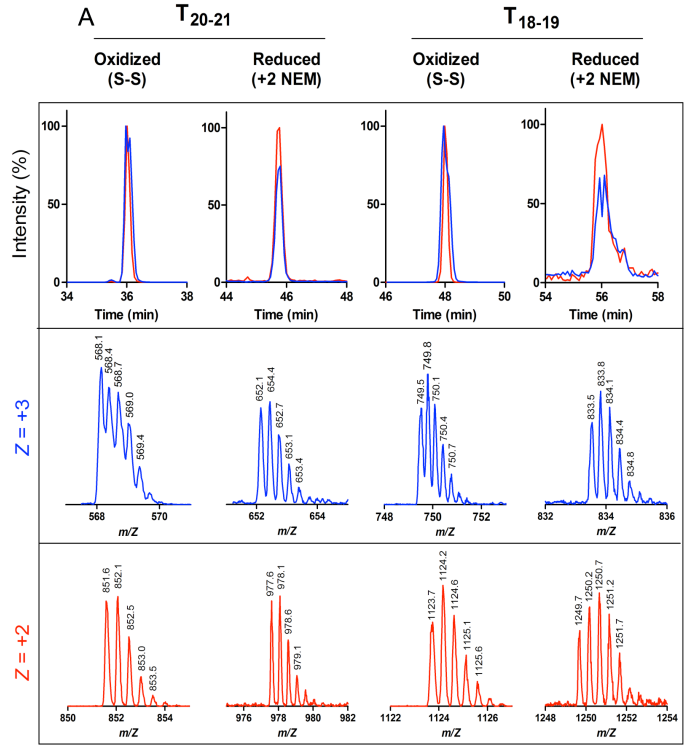


Fig. S4. *In-gel* digestion-HPLC-MS and redox analysis of DksA. (A) Cysteine containing peptides, T_{18-19} and T_{20-21}, in both oxidized or reduced states were analyzed by HPLC-Enhanced Resolution MS. Upper panel shows a representative extracted ion current chromatogram for *Z* = +2 (red) and *Z* = +3 (blue) corresponding ions for each peptide. *Enhanced resolution* mass spectra for each ion are shown in lower panels. (B) Redox state of thiol groups in DksA cysteine residues in anaerobic, log phase *Salmonella* was evaluated by Western blot analysis after derivatization with the alkylation agent AMS as described in **Fig. S3**. Some of the bacterial cultures were treated for the indicated times with 1 or 10 μM H_2O_2.



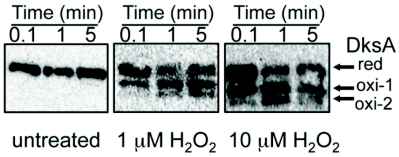


Fig. S5. Characterization of *Salmonella* strains expressing *dnaJ* variants. (A) Growth of WT, Δ*dnaJ*, and complemented *Salmonella* strains in LB broth at 37 or 45°C. (B) Growth of WT, Δ*dksA*, and Δ*dnaJ* *Salmonella* in EG minimal media as determined spectrophotometrically by following OD_{600}. (C) Immunoblot of DksA-3xFLAG and DnaK in lysates (20 μg/lane) obtained from the indicated *Salmonella* strains grown to log phase in LB broth. Select cultures (+) were treated with 1 mM H_2O_2 for 30 min. (D) DnaJ expression in the indicated *Salmonella* strains grown to exponential phase in LB broth as determined by immunoblot analysis. DnaK is shown for comparison. The results are representative of 2-3 independent experiments.

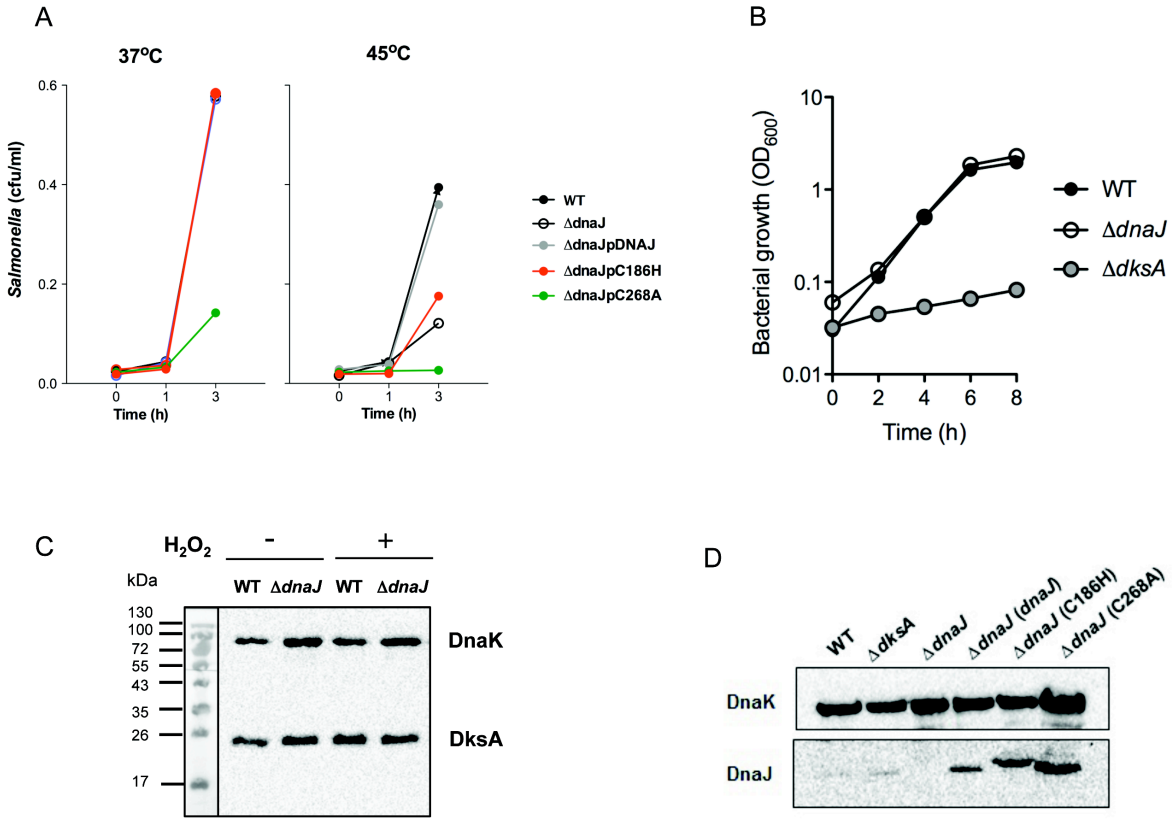


Fig. S6. Transcription of *hisG* in *Salmonella* and purification of RNA polymerase from *Salmonella*. (A and B) Abundance of *hisG* transcripts in anaerobically grown *Salmonella* left untreated or exposed to 1 or 10 μM H_2O_2. The abundance of *hisG* transcripts was normalized to the housekeeping gene *rpoD*. ***p<0.001 compared to untreated controls as determined by One-way ANOVA. Data are expressed as mean +/- SD from 4 independent experiments. (C) Growth of anaerobic *Salmonella* in E salts minimum media supplemented with glucose and casamino acids in the presence of 1 or 10 μM H_2O_2. (D) Purified *Salmonella* RNA polymerase holoenzyme was examined by 8% SDS-PAGE and Coomassie Brilliant Blue staining. *E. coli* RNA polymerase holoenzyme was included for comparison.

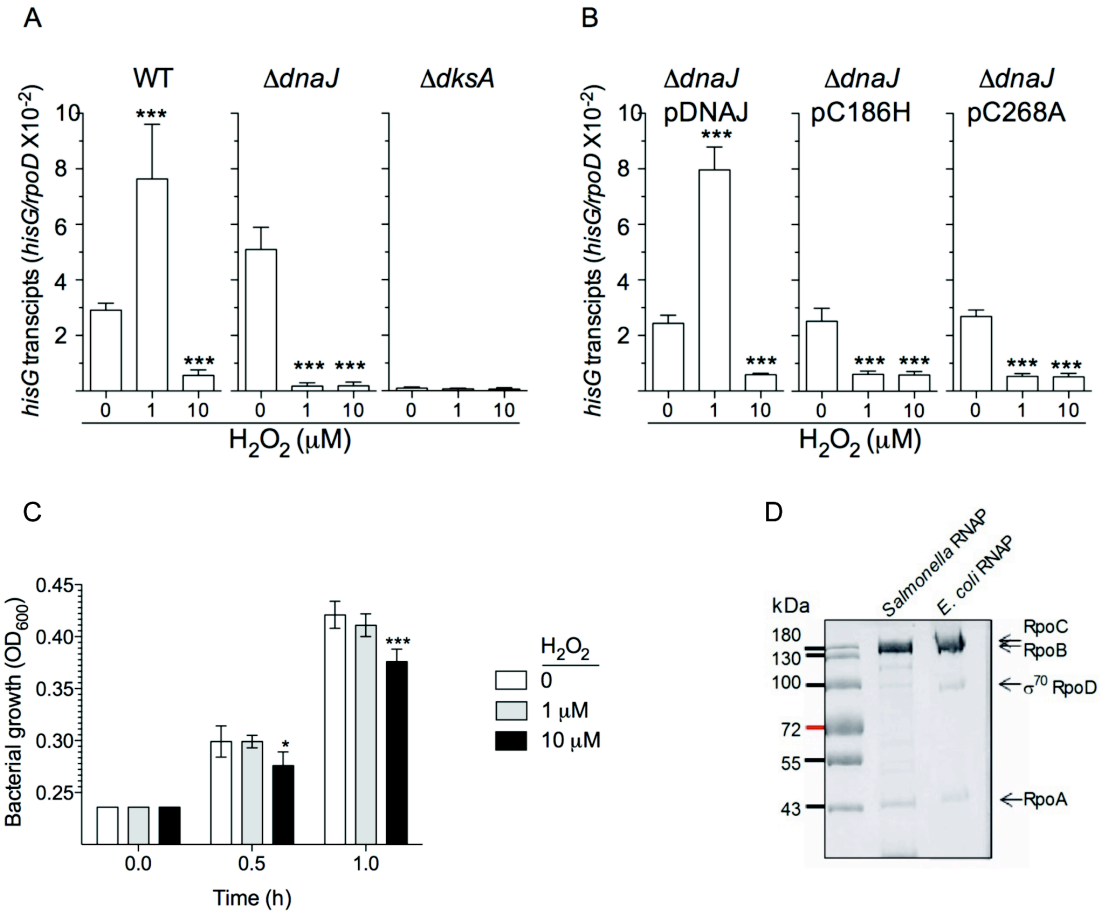
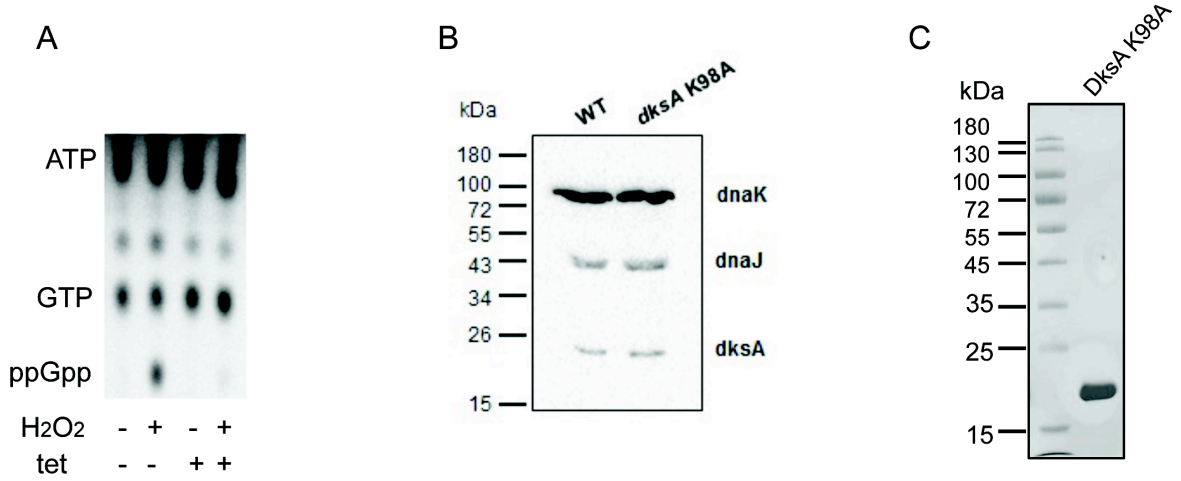


Fig. S7. Effects of tetracycline on H_2O_2-stimulated ppGpp synthesis. (A) TLC autoradiogram of ^{32}P-labeled nucleotides in WT *Salmonella* treated with 70 μg/ml tetracycline for 3 min before the addition of 25 μM H_2O_2 for 1 min. The blot shown is representative of 2 independent experiments. (B) DksA-3xFLAG, DnaJ and DnaK proteins were examined by immunoblot in the indicated *Salmonella* strains that had been grown to log phase. (C) Recombinant DksA K98A protein used in **Fig. 6G**.



References:

1. Radi R, Beckman JS, Bush KM, & Freeman BA (1991) Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. *J Biol Chem* 266:4244-4250.

2. Fitzsimmons LF, Liu L, Kim JS, Jones-Carson J, & Vazquez-Torres A (2018) *Salmonella* Reprograms Nucleotide Metabolism in Its Adaptation to Nitrosative Stress. *MBio* 9: e00211-18.

3. Henard CA, Bourret TJ, Song M, & Vazquez-Torres A (2010) Control of redox balance by the stringent response regulatory protein promotes antioxidant defenses of *Salmonella*. *J Biol Chem* 285:36785-36793.

4. Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166:557-580.

5. Henard CA*, et al.* (2014) The 4-cysteine zinc-finger motif of the RNA polymerase regulator DksA serves as a thiol switch for sensing oxidative and nitrosative stress. *Mol Microbiol* 91:790-804.

6. Smith DB & Johnson KS (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. *Gene* 67:31-40.

7. Datsenko KA & Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A* 97:6640-6645.

8. Tapscott T*, et al.* (2018) Guanosine tetraphosphate relieves the negative regulation of *Salmonella* pathogenicity island-2 gene transcription exerted by the AT-rich ssrA discriminator region. *Sci Rep* 8:9465.

9. Wang RF & Kushner SR (1991) Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* 100:195-199.

10. Song M, Kim JS, Liu L, Husain M, & Vazquez-Torres A (2016) Antioxidant Defense by Thioredoxin Can Occur Independently of Canonical Thiol-Disulfide Oxidoreductase Enzymatic Activity. *Cell Rep* 14:2901-2911.