Supporting Information

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SI Text: Plasmid and Strain Constructions

Oligonucleotides used in constructing strains and plasmids are listed in Table S2. Chromosomal alleles were moved by P1 transductions. Chromosomal deletions by inserting antibiotic resistance markers were done in BW25113 by the method of Datsenko and Wanner, and subsequently moved into various other strains by P1 transduction and selected for antibiotic resistance (1). Elimination of the antibiotic resistance markers created by the method of Datsenko and Wanner was performed as described and carried out after introducing the deletion allele with the antibiotic resistance marker into any strain (1). The gene for DsbA was deleted as published (2). *dsbB*::K_m^R in HK329 was created as in (3) and *dsbB*::Cm^R in MER144 using oligonucleotides dsbBH43 and dsbBcatP2 and template pKD3. *dsbC*::K_m^R was created using primers dsbCH1 and dsbCH2 and template pKD4. For *dsbD*::K_m^R, the primers dsbDH1 and dsbDH2 and template pKD13 were used. The *gshA*::Kan^R and *gshA*::Cm^R alleles were derived from MJF152 and MJF141, respectively. Primers cydDH1 and cydDH2 using pKD13 were used to create a *cydD*::K_m^R allele. *mdlA*::K_m^R was made using primers mdlA5′P4 and mdlA3′P1 and template pKD13. The *ggt*::Kan^R allele was constructed using primers ggt-kan-P2 and ggt-del-P1. Strains were made *phoR*$^-$ by P1 transduction using a lysate derived from DHB73, selecting for K_m^R and screening for blue colonies on media plates containing XP (5-bromo-4-chloro-3-indolyl phosphate).

Oligonucleotides ssTorT-for and ssTorT-rev coding for the signal sequence of TorT were annealed and ligated into the *Nco*I site of pDSW204, resulting in pMER81. Plasmid pMER79 was created by cloning the *Xba*I and *Hin*dIII-flanked 3xFLAG sequence derived from pNB100 (4) into the *Xba*I and *Hin*dIII sites of pMER81. The genes for TrxA, TrxC, GrxA, and GrxC were amplified from MG1655 using oligonucleotides (trxA-*Bsp*HI-for, trxA-ns-*Xba*I-rev, trxC-*Nco*I-for, trxC-ns-*Xba*I-rev, grxA-*Bsp*HI-for, grxA-ns-*Xba*I-rev, grxC-*Nco*I-for, grxC-ns-*Xba*I-rev) digested with either *Bsp*HI or *Nco*I and *Xba*I and cloned into the *Nco*I and *Xba*I sites of pMER79 to create pMER90, pMER91, pMER92, and pMER94, respectively. pMER96 was generated by amplifying the bacteriophage T4 glutaredoxin 1 gene *nrdC* from pGRX1 using the oligonucleotides AflIII-NrdC-for and nrdC-ns-*Xba*I-rev and cloning the resulting DNA fragment into the *Nco*I and *Xba*I sites of pMER79. The glutaredoxin 3 gene (*grxC*) was amplified by PCR from purified *E. coli* chromosomal DNA with primers Sal-grxC-F and R-grxC-Hind. The PCR product was digested with *Sal*I and *Hin*dIII, ligated into *Sal*I- and *Hin*dIII-digested pTrc99a-ssTorA-TrxA resulting in pTrc99a-ssTorA-GrxC. GrxC active site mutants were constructed as follows: *grxC* was amplified from plasmid pTrc99a-ssTorA-grxC with forward primers Sal-grxC[AA] for GrxC[AA], Sal-grxC[CA] for GrxC[CA], Sal-grxC[AC] for GrxC[AC] and with R-grxC-Hind as the reverse primer for all three constructs. Each PCR product was digested with *Sal*I and *Hin*dIII, then ligated into *Sal*I- and *Hin*dIII-digested pTrc99a-ssTorA-TrxA, resulting in pTrc99a-ssTorA-GrxC[AA], pTrc99a-ssTorA-GrxC[CA], and pTrc99a-ssTorA-GrxC[AC]. TrxA active site mutants were constructed according to the Quickchange method (Stratagene) using pTrc99a-ssTorA-TrxA as template and the oligonucleotides trxA-AA-top and trxA-AA-bott to create pMER163, trxA-CA-top and trxA-CA-bott, creating pMER164, trxA-AC-top and trxA-AC-bott, resulting in pMER165. The C65Y mutation in GrxC[CA] was introduced by site-directed mutagenesis according to the Quickchange protocol (Stratagene) using primers GrxC-C66Y-for and GrxC-C66Y-rev amplifying plasmid pTrc99a-ssTorA-GrxC[CA] and the resulting isolated plasmid was verifying by sequencing. The plasmid pMER187 was created by subcloning the *grxC[CA]C65Y* gene from the site-directed mutagenesis using *Xba*I and *Hin*dIII restriction sites into *Xba*I/*Hin*dIII-digested pTrc99a-ssTorA-GrxC. The genes for the DsbA substrates LivK (leucine-specific-binding protein), YodA (calcium-induced cadmium-binding protein), and RcsF (sensory component of the Rcs phosphorelay system) were subcloned from plasmids pHK641, pHK642, and pHK646 by excising a DNA fragment containing the genes and a 3′ *c-Myc* sequence using *Kpn*I and *Xba*I and cloning these into the *Kpn*I/*Xba*I sites of pBAD42, resulting in the plasmids pMER154, pMER155, and pMER156, respectively.

1. Datsenko KA, Wanner BL (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci USA* 97:6640–6645.
2. Kadokura H, Tian H, Zander T, Bardwell JC, Beckwith J (2004) Snapshots of DsbA in action: detection of proteins in the process of oxidative folding. *Science* 303:534–537.
3. Kadokura H, Bader M, Tian H, Bardwell JC, Beckwith J (2000) Roles of a conserved arginine residue of DsbB in linking protein disulfide-bond-formation pathway to the respiratory chain of Escherichia coli. *Proc Natl Acad Sci USA* 97:10884–10889.
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Table S1. *E. coli* strains and plasmids

Strain or plasmid	Genotype or description	Ref. or source
Strains		
MC1000	*araD*139 Δ(*araABC-leu*)7697 *galU galK* Δ(*lac*)X74 *rpsL thi*	1
MG1655	*E. coli* K12	2
DHB3	Δ(*ara-leu*)7697 *araD*139 Δ*lacX*74 *galE galK rpsL phoR* Δ(*phoA*)PvuII Δ*malF*3 *thi*	3
DHB73	DHB3 $phoA^+$ $phoR^-$ *proC*::*Tn10*	Dana H. Boyd
DH5α	F^- Φ80*lacZ*ΔM15 Δ(*lacZYA-argF*)U139 *recA*1 *endA*1 *hsdR*17 (r_K^- m_K^-) *gal*$^-$ *phoA sup*E11 λ$^-$ *thi*$^-$1 *gyrA*96 *relA*1	Invitrogen
BW25113	*lacI*^{q} $rrnB_{T17}$ Δ$lacZ_{WJ16}$ *hsdR*514 Δ$araBAD_{AH33}$ Δ$rhaBAD_{LD78}$	4
MJF141	DHB4 *gshA*::Cm^R	Melinda Faulkner
MJF152	DHB4 *gshA*::Km^R	5
HK295	MC1000 Δ*ara*714 *leu*$^+$	6
HK317	HK295 Δ*dsbA*	7
HK329	HK295 Δ*dsbA* Δ*dsbB*	This study
HK581	HK317 Δ*gshA*	This study
HK582	HK329 Δ*gshA*	This study
HK453	HK295 Δ*dsbA* Δ*dsbC* Δ*dsbD*	This study
MER144	HK453 Δ*dsbB*	This study
MER380	HK453 Δ*gshA*	This study
MER360	HK295 *phoR*$^-$..*proC*::*Tn10*	This study
MER390	HK317 *phoR*$^-$..*proC*::*Tn10*	This study
MER392	HK329 *phoR*$^-$..*proC*::*Tn10*	This study
MER382	MER360 Δ*gshA*	This study
MER394	HK581 *phoR*$^-$..*proC*::*Tn10*	This study
MER396	HK582 *phoR*$^-$..*proC*::*Tn10*	This study
MER554	MER360 Δ*mdlA*	This study
MER556	MER390 Δ*mdlA*	This study
MER471	MER360 Δ*ggt*	This study
MER496	MER392 Δ*ggt*	This study
HK408	HK295 Δ*cydD*	This study
HK498	HK317 Δ*cydD*	This study
MER487	HK408 *phoR*$^-$..*proC*::*Tn10*	This study
MER491	HK498 *phoR*$^-$..*proC*::*Tn10*	This study
Plasmids		
pCP20	pINT-ts *araC* P_{araB}-γ-β-*exo*	4
pKD46	pINT-ts *araC* P_{araB}-γ-β-*exo-tL3*	4
pKD3	pANTS-γ FRT flanked cat^R	4
pKD4	pANTS-γ FRT flanked kan^R	4
pTrc99a	ori_{pBR322} P_{trc} T_{rrnB} *bla*	Promega
pDSW204	pTrc99a with attenuated promoter	8
pAM239	ori_{pSC101} P_{lac} $spec^R$	laboratory collection
pBAD42	ori_{pSC101} *araC* P_{araB} $spec^R$	L.M. Guzman
pNB100	pBAD18 $FLAG_3$	9
pGRX1	pQE60-*nrdC*	10
pHK641	pAM238 *livK-c-Myc*	7
pHK642	pAM238 *yodA-c-Myc*	7
pHK646	pAM238 *rcsF-c-Myc*	7
pMER79	pDSW204-TorTss-FLAG	This study
pMER81	pDSW204-TorTss	This study
pMER90	pDSW204-TorTss-*trxA*-FLAG	This study
pMER91	pDSW204-TorTss-*trxC*-FLAG	This study
pMER92	pDSW204-TorTss-*grxA*-FLAG	This study
pMER94	pDSW204-TorTss-*grxC*-FLAG	This study
pMER96	pDSW204-TorTss-*nrdC*-FLAG	This study
pTrc99a-ssTorA-TrxA	pTrc99a TorAss-*trxA*	11
pMER163	pTrc99a TorAss-*trxA* (C32A, C35A mutations in *trxA*)	This study
pMER164	pTrc99a TorAss-*trxA* (C35A mutation in *trxA*)	This study
pMER165	pTrc99a TorAss-*trxA* (C32A mutation in *trxA*)	This study
pTrc99a-ssTorA-GrxC	pTrc99a TorAss-*grxC*	This study
pTrc99a-ssTorA-GrxC[AA]	pTrc99a TorAss-*grxC* (C11A C14A mutations in *grxC*)	This study
pTrc99a-ssTorA-GrxC[CA]	pTrc99a TorAss-*grxC* (C14A mutation in *grxC*)	This study

Strain or plasmid	Genotype or description	Ref. or source
pTrc99a-ssTorA-GrxC[AC]	pTrc99a TorAss-*grxC* (C11A mutation in *grxC*)	This study
pMER187	pTrc99a TorAss-*grxC* (C14A C65Y mutations in *grxC*)	This study
pMER154	pBAD42 *livK-c-Myc*	This study
pMER155	pBAD42 *yodA-c-Myc*	This study
pMER156	pBAD42 *rcsF-c-Myc*	This study

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Table S2. Oligonucleotides

Name	Sequence (5′→3′)
dsbBH43	aaatatagcggcaggaaaaaagcgctcccgcaggagcgccgaatggattagtgtaggctggagctgcttc
dsbBcatP2	aatgaattggtttaaactgcgcactctatgcatattgcagggaaatgattcatatgaatatcctcctta
dsbCH1	atcaacagcatcacccgcgggcgtgatgtctgaaaagaacgggaagatttgtgtaggctggagctgcttcg
dsbCH2	acggcgacgaaggtgtatctgttgtttcacgcgaattatttaccgctggtcatatgaatatcctccttag
dsbDH1	ccggaacttctggttttacctgttacacacggagacacagattacctctcattccggggatccgtcgacc
dsbDH2	tcttcacgttgcacgctattttcctccgtctttcccactgcaagtgtcgtgtgtaggctggagctgcttc
cydDH1	tgtaacattgctctgcaaataattctgataactcacctgctaagcgtgcaattccggggatccgtcgacc
cydDH2	atccatttatgacgtttatacagtgccagatagggtagcaaagcgcgcatgtgtaggctggagctgcttc
mdlA5′P4	tctttacccatcgaataaatatccagaatcaggtcaggacacaacgcgtgattccggggatccgtcgacc
mdlA3′P1	gcttgagagtcggccacagttggctaaaactacgcatcgacggcctcctctgtaggctggagctgcttcg
ggt-kan-P2	ccgacgtttttacgccgggtggccattgctgctctgctctcaggaagttgcatatgaatatcctccttag
ggt-del-P1	cgccgttaaatcatccaccgagcgcgggtcggatgcgccgtacaactcacgtgtaggctggagctgcttc
ssTorT-for	tcatgcgcgtactgctatttttacttctttccctttcatgttgccggcattttccatgg
ssTorT-rev	catggaaaatgccggcaacatgaaaagggaaagaagtaaaaatagcagtacgcg
trxA-BspHI-for	actatcatgagcgataaaattattcacctgac
trxA-ns-XbaI-rev	gctctagacgccaggttagcgtcgaggaac
trxC-NcoI-for	catgccatgggtaataccgtttgtacccattgtcagg
trxC-ns-XbaI-rev	gctctagaaagagattcgttcagccagc
grxA-BspHI-for	actatcatgatgcaaaccgttatttttggtcgttcg
grxA-ns-XbaI-rev	gctctagaggcgtccagattttctttcac
grxC-NcoI-for	catgccatggccaatgttgaaatctataccaaagaaac
grxC-ns-XbaI-rev	gctctagatttcagcaggggatccagtcc
AflIII-NrdC-for	ttacatgtttaaagtatatggttatgatagc
nrdC-ns-XbaI-rev	gctctagatttaaagtattcccgcaattg
Sal-grxC-F	ctctcgtcgacatggccaatgttgaaatctatacca
R-grxC-Hind	ctctcaagcttatttcagcaggggatccagt
Sal-grxC[AA]	ctctcgtcgacatggccaatgttgaaatctataccaaagaaaccgcgccgtatgcgcatcgtgcaaaagcactgctg
Sal-grxC[CA]	ctctcgtcgacatggccaatgttgaaatctataccaaagaaacctgcccgtatgcgcatcgtgcaaaagcactgctg
Sal-grxC[AC]	ctctcgtcgacatggccaatgttgaaatctataccaaagaaaccgcgccgtattgccatcgtgcaaaagcactgctg
R-grxC-Hind	ctctcaagcttatttcagcaggggatccagt
GrxC-C66Y-for	cacagcacattggcggctacgatgacttgtatgcattg
GrxC-C66Y-rev	caatgcatacaagtcatcgtagccgccaatgtgctgtg
trxA-AA-top	cgatttctgggcagagtgggccggtccggccaaaatgatcgccccgattc
trxA-AA-bott	gaatcggggcgatcattttggccggaccggcccactctgcccagaaatcg
trxA-CA-top	cgatttctgggcagagtggtgcggtccggccaaaatgatcgccccgattc
trxA-CA-bott	gaatcggggcgatcattttggccggaccgcaccactctgcccagaaatcg
trxA-AC-top	cgatttctgggcagagtgggccggtccgtgcaaaatgatcgccccgattc
trxA-AC-bott	gaatcggggcgatcattttgcacggaccggcccactctgcccagaaatcg