Supporting Information

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SI Materials and Methods

Purification of QseE-His and Reconstitution into Liposomes. A total of 500 mL of NR33 cells was grown in LB medium with the appropriate antibiotic at 37 °C to anf optical density at 600 nM (OD_{600}) of 0.3, and induced with 0.5 M IPTG. Cells were grown for 4 more hours at 37 °C, then harvested by centrifugation at 10,000 × *g* for 15 min. Inclusion bodies were isolated by resuspending the cells in 10 mL of BPERII Bacterial Protein Extraction Reagent (Pierce) and allowed to shake for 10 min. Cells were centrifuged at 10,000 × *g* for 15 min, and the pellet was resuspended in 10 mL of BPERII. Lysozymes to a final concentration of 200 μg/mL and 50 μL of His-tag protease inhibitor mixture (Sigma) were added, and cells were incubated at room temperature for 10 min. A total of 50 mL of diluted BPERII (1:20) was added to sample, and then cells were centrifuged for 15 min at 10,000 × *g*. Cells were washed again with 50 mL of diluted BPERII and spun. Cells were washed a total of 3 times, and each time the supernatant was saved for analysis. The final pellet contained purified inclusion bodies and QseE-His. Liposomes were reconstituted as described previously (1, 2).

Phosphorylation of QseE-His in Liposomes. A total of 20 μL of the QseE-His-loaded liposomes was adjusted to 10 mM $MgCl_2$, 1 mM DTT, and various concentrations of signal molecules. Liposomes were frozen and thawed 3 times in liquid nitrogen, and kept light protected at room temperature for 1 h, allowing the loading of signals and the reformation of the liposomes. [γ^{32}P]dATP (0.5 μL; 110 TBq/mmol) was added to each reaction, and tubes were incubated for 5 min. After 5 min, 20 μL of SDS-loading dye containing 20% SDS was added to each reaction, samples were subject to SDS/PAGE analysis without boiling, and phosphorylation was visualized by using a Storm PhosphorImager. Bands were quantified by using IMAGEQUANT version 5.0 software (Amersham Pharmacia).

Identification of Proteins by Mass Spectrometry. Kinase assays were completed as detailed above, but γ^{32}ATP was replaced with cold ATP. Samples were subjected to SDS/PAGE, and gels were stained with Coomassie stain. Bands corresponding to the size of bands from autoradiography detailed above were excised and submitted to the University of Texas Southwestern Protein Chemistry Core Facility. Briefly, in-gel bands were subject to trypsin digestion and reversed-phase, non-high-performance liquid chromatography/ion trap mass spectrometry. Resulting data sets were used to search against the EHEC EDL933 genome.

RNA Extraction. Cultures of 8624, NR01, NR02, and NR03 were grown aerobically in LB medium at 37 °C overnight and then were diluted 1:100 in low-glucose red DMEM and allowed to grow at 37° until they reached OD_{600} of 1.0. RNA was extracted from 3 replicates of each strain by using a RiboPure bacterial RNA isolation kit (Ambion) according to the manufacturer's instructions.

Real-Time qRT-PCR. Primers used in qRT-PCR analysis were designed by using Primer Express v 1.5 (Applied Biosystems) and are listed in Table S2. qRT-PCR analysis was conducted by using Applied Biosystems ABI 7500 sequence detection system using a 1-step reaction. Each primer set was checked for amplification efficiency by standard curves resulting from using varying concentrations of RNA template. To ensure template specificity, products were heated to 95 °C for 15 seconds, cooled to 60 °C, and heated to 95 °C while fluorescence was monitored. To analyze gene expression in NR01, NR02, and NR03 compared with 86–24 *Escherichia coli*, relative quantification analysis was used. Parameters for cDNA generation and amplification were as follows: 1 cycle at 48 °C for 30 min, 1 cycle at 95 °C for 10 min, and 40 cycles at 95° for 15 seconds and 60 °C for 1 min. The RNA polymerase subunit Z, *rpoZ*, was used as an endogenous control. In each reaction of 20 μL, 10 μL of 2× SYBR master mix, 0.1 μL of Multiscribe reverse transcriptase (Applied Biosystems), and 0.1 μL of RNase inhibitor (Applied Biosystems) were added.

Detection, Quantification, and Statistical Analysis. Applied Biosystems ABI Sequence Detection 1.3 software was used for initial collection of data. Values were normalized to *rpoZ* and analyzed by using the comparative critical threshold (C_T) value as previously described (3). Expression is shown in graphs as *n*-fold change in expression level compared with wild-type levels at late exponential growth. Error bars represent the standard deviations of the $\Delta\Delta C_T$ value. The Student *t* test was performed to assess statistical significance. A *P* value of less than 0.05 was considered significant.

Membrane Preparation and Sucrose Density Gradient Centrifugation. Membrane separation methodology was adapted from previously published methods for isolation of outer membranes from Gram-negative bacteria (4–6). A total of 500 mL of 86–24 and NR05 were grown in LB at 37 °C until they reached OD_{600} of 0.2. Cells were induced by the addition of 0.2% arabinose and harvested once they reached OD_{600} of 1.0 by centrifugation at 10,000 × *g* for 15 min. Cells were resuspended in 10 mL of 0.75 M sucrose in 5 mM Tris, pH 7.5. While stirring on ice, 40 mL of 10 mM EDTA-tetrasodium in 5 mM Tris, pH 7.5, was added, and cells were incubated on ice while stirring for 30 min. Lysozyme was added to a final concentration of 200 μg/mL, and cells were incubated while stirring at room temperature for 30 min. Cells were osmotically lysed by the addition of 4.5 volumes or 240 mL of ice-cold dH_2O, and they were incubated on ice for 30 min while swirling. Debris was collected by centrifuging cells at 10,000 × *g* for 30 min. To collect total membranes, supernatants from the previous spin were divided into 8 tubes and ultracentrifuged for 2 h at 200,000 × *g* at 4 °C. Total membrane pellets were resuspended each in 1 mL of resuspension solution [25% sucrose in 5 mM Tris, 30 mM $MgCl_2$, 1 EDTA-free MiniComplete (Bio-Rad), 15 μL of Benzonase (Invitrogen), and 13 μL of His-tag protease inhibitor mixture (Sigma)]. Tubes were incubated, shaking at room temperature for 30 min to degrade DNA. Aliquots were taken, and total protein per membrane prep was determined by using the Bradford assay (Bio-Rad). Linear sucrose gradients were poured in 14 × 99 mm Beckman ultracentrifuge tubes by layering 1.8 mL each of 55%, 50%, 45%, 40%, 35%, and 30% sucrose, and crude membrane preparations were layered on top of the 30% sucrose. Sucrose gradients were ultracentrifuged overnight for 17 h in an SW-40 Beckman rotor at 256,000 × *g* at 4 °C. Approximately 20 fractions of 500 μL each were collected from each sucrose gradient by puncturing the bottom of the tube with an 18-gauge needle and letting fractions leave the tube by gravity flow. The refractive indexes of each fraction were determined by using a refractometer (Fisher Scientific), and the density of each fraction in grams per milliliter was calculated based on the refractive index (7). Fractions were diluted in SDS-loading dye, subjected to SDS/PAGE, and immunoblotted as described above to detect QseG.

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Table S1. Strains and plasmids used in this study

Plasmid or strain	Relevant genotype	Reference or source
Strains		
86–24	Stx2 and EHEC strain (serotype O157:H7)	(1)
NR01	86–24 qseE nonpolar mutant	(2)
NR02	86–24 qseF nonpolar mutant	(2)
NR03	86–24 qseG nonpolar mutant	This study
NR04	NR01 complemented with plasmid pNR01	(2)
NR05	NR03 complemented with plasmid pNR03	This study
NR06	NR02 complemented with plasmid pNR02	(2)
KRL7	86–24 qseE polar mutant	(2)
NR26	pK187 in NR02	(2)
NR27	pKC471 in NR02	(2)
NR28	pKH35-4 in BL21 DE3 cells	(3)
NR30	pNR15 in NR02	(2)
NR31	pET16 in NR02	(2)
NR32	p635 in NR02	(2)
NR33	pNR30 in BL21 DE3 cells	This study
NR34	pcya in 8624	This study
NR36	pcya in $qseG^-$	This study
NR37	pcya-tir in 8624	This study
NR39	pcya-tir in $qseG^-$	This study
NR41	pcya in $qseG^+$	This study
NR43	pcya-tir in $qseG^+$	This study
NR44	pDE2GFP in 8624	This study
NR45	pDE2GFP in $qseG^-$	This study
NR46	pDE2GFP in $qseG^+$	This study
CVD451	86–24 escN mutant	(4)
UMD872	E2348/69 espA mutant	(5)
Plasmids		
pBadMycHis	C-terminal Myc-His-tag cloning vector	Invitrogen
pRS551	lacZ reporter gene fusion vector	(6)
pNR01	qseE in pBadMycHis	(2)
pVS254	qseE-lacZ in pRS551	This study
pNR02	qseF in pBadMycHis	(2)
pNR03	qseG in pBadMycHis	This study
pKD3	pANTSγ derivative containing FRT-flanked chloramphenicol resistance	(7)
pKM201	λ red recombinase expression plasmid	(8)
pCP20	TS replication and thermal induction of FLP synthesis.	(7)
pRS551	lacZ reporter gene fusion vector	(6)
pNR10	espFu in pRS551	(2)
pNR30	pET21 + qseE	This study
pKH35–4	yfhA in pET21a(+)	(3)
pNR15	qseF in pACYC177	(2)
pACYC177	Cloning vector	New England Biolabs
pVS262	espFu in TOPO	(2)
Topo	Commercial blunt-end cloning vector	Invitrogen
pET16	Expression vector	Novagen
pET21	Expression vector	Novagen
pCya	cyaA of Bordetella pertussis cloned into pACYC177	(9)
pCya-Tir	tir-cyaA fusion cloned into pACYC177	(9)
pDE2GFP	gfp vector driven by the lac promoter	Clontech

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Table S2. Oligonucleotides used in this study

Primer name	Sequence	Description
QseE RTR	CGC GCC ATG ATC TTC GA	Real-time analysis
QseE RTF	CCC TTC ACC GCC CCT TT	Real-time analysis
QseF RT R	CGT AAG CTG CTG CAA ATT ACC A	Real-time analysis
QseF RT F	CGC CCC GCC ATT CTC	Real-time analysis
YfhG-F-Bad	GGT ACC CAA CGA TTA TTG CCC CGA CG	Construction of pNR03
YfhG-R-Bad	GAA TTC CCG ACC TCA TGG GTG GAT GGC GCG	Construction of pNR03
yfhG Red P1	ATC CTC ACG TAC GAC ATG TAC GCT CCG GTT TCT CCG CGC TGT CCA TGT CCG TGT AGG CTG GAG CTG CTT C	Construction of the qseG isogenic mutant
yfhG Red P2	TCC AGT TGC TGC TGT AGA ACG TGA TGT TGC TGG CGC AAT GTA TCC AGC TCC ATA TGA ATA TCC TCC TTA	Construction of the qseG isogenic mutant
QsePet21 F-final	CGA ATG ACG CAC GGA TCC GAG CCT GCC GT	Construction of pNR30
QseERpET21	GGG ATA GGC TGT GCG GCC GCT TTC GTG TTT	Construction of pNR30
RpoZ RTF	TGC AGG TAG GCG GAA AGG	Real-time analysis
rpoZRTR	GCG CAG CGC GAT TAC AGT	Real-time analysis
eae RTF	GCT GGC CCT TGG TTT GAT CA	Real-time analysis
eae RTR	GCG GAG ATG ACT TCA GCA CTT	Real-time analysis
espA RTF	TCA GAA TCG CAG CCT GAA AA	Real-time analysis
espA RTR	CGA AGG ATG AGG TGG TTA AGC T	Real-time analysis
QseG RTF	TGC GCA GGC ATT AAA CGA T	Real-time analysis
QseG RTR	CTG CGG GCG ATG GAT TGT GCT	Real-time analysis
ler RTF	CGACCAGGTCTGCCC	Real-time analysis
ler RTR	GCGCGGAACTCATC	Real-time analysis
glnBRTF	CACTGGCTGAAGTCGGCATT	Real-time analysis
glnBRTR	TGGCGGCCAAAACCTTT	Real-time analysis