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Perturbation of the Oxidizing Environment of the Periplasm Stimulates the PhoQ/PhoP System in *Escherichia coli*

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The PhoQ/PhoP two-component system is repressed by divalent cations, such as Mg^{2+} and Ca^{2+}, in the growth medium and stimulated by low pH and certain cationic antimicrobial peptides. In *Escherichia coli*, it was recently shown that the histidine kinase PhoQ is also modulated by at least two additional factors, the small membrane proteins SafA and MgrB. This raises the possibility that the PhoQ/PhoP circuit has additional regulatory components and integrates additional input signals. We screened *E. coli* transposon insertion mutants to look for proteins that modulate the activity of the PhoQ/PhoP system, and we uncovered a role for DsbA, a periplasmic oxidant that facilitates the formation of disulfide bonds. Deletion of *dsbA* or *dsbB*, which maintains a pool of oxidized DsbA, leads to increased transcription of at least two PhoP-regulated genes. Addition of the reducing agent dithiothreitol to wild-type cells had a similar effect, and treatment of a *dsbA* null strain with the oxidant Cu^{2+} rescued the reporter gene expression phenotype. We also demonstrated that expression of an MgrB mutant that lacked cysteines blocked the effect of a *dsbA* null mutation on PhoQ/PhoP activity, suggesting that MgrB acts downstream of DsbA in this pathway. Taken together, these results demonstrate that a decrease in the oxidizing activity of the periplasm stimulates PhoQ/PhoP and may reveal a new input stimulus for this important two-component system.

Many two-component signaling systems depend on more than their two core protein components, the histidine kinase and response regulator, for proper signal transduction. Additional proteins may modulate various steps in signaling pathways, such as input sensing, phosphoryl transfer, dephosphorylation, or output regulation. Some of these auxiliary proteins have well-understood functions, such as detecting specific input signals and relaying this information to the sensory domain of a histidine kinase, or connecting two otherwise distinct circuits (1, 7, 14, 20). However, in many cases the physiological significance of these proteins remains poorly understood.

One example of a well-studied two-component system whose activity is modulated by additional protein components is the PhoQ/PhoP system, which is found in *Escherichia coli*, *Salmonella* species, and related bacteria. The sensor kinase PhoQ controls the phosphorylation state of PhoP through PhoQ autophosphorylation, followed by phosphoryl transfer to PhoP and PhoQ-mediated phosphatase activity against phosphorylated PhoP (PhoP-P). Thus, the level of PhoP-P is set by the balance between the kinase and phosphatase activities of PhoQ. For simplicity, we refer to conditions that increase or decrease the ratio of PhoQ kinase to phosphatase activity, which lead to increased or decreased PhoP-P, as stimulating or repressing PhoQ activity, respectively. PhoQ activity is repressed by growth medium containing high concentrations of Mg^{2+} and is stimulated by low pH and certain cationic antimicrobial peptides (4, 16, 25, 28). Recent work also uncovered two small membrane proteins that modulate PhoQ activity: SafA (B1500) (10), which to date has only been identified in *E. coli*, and MgrB, which is found in numerous *Enterobacteriaceae* (18). SafA stimulates PhoQ and functions as a connector between the EvgS/EvgA and PhoQ/PhoP two-component systems (10). MgrB, on the other hand, represses PhoQ activity and, as it is part of the PhoP regulon, is part of a negative feedback loop (18). The function of this feedback loop, however, is not understood.

A protein that regulates a signal transduction pathway may provide an entry point for additional input signals, since factors that modulate the protein's activity will modulate the circuit output. We therefore looked for additional factors that affect PhoQ/PhoP signaling by screening *E. coli* transposon-insertion mutants for increased expression of a PhoP-regulated reporter gene. From this screen we found that disruption of pathways associated with disulfide bond formation in the periplasm leads to activation of the PhoQ/PhoP system.

Disulfide bond formation in the periplasm is catalyzed by the periplasmic protein DsbA and the membrane protein DsbB (reviewed in references 13, 15, and 23). DsbA oxidizes cysteine residues to form disulfide bonds through a thiol-disulfide exchange reaction. The reduced form of DsbA resulting from this reaction is reoxidized by the membrane protein DsbB. We found that deletion of *dsbA* results in activation of several PhoP-regulated genes in a PhoQ-dependent manner. In addition, deletion of *dsbB* as well as treatment with the reducing agent dithiothreitol (DTT) has a similar effect on PhoQ/PhoP signaling. Furthermore, we provide evidence suggesting this redox-sensitive pathway acts through MgrB.

To our knowledge, these results are the first demonstration that disruption of the oxidizing environment of the periplasm stimulates PhoQ. The results also raise the possibility that the periplasmic redox state may be a physiologically important input stimulus for the *E. coli* PhoQ/PhoP system whereby reducing conditions or possibly other factors that affect disulfide bonding sta-

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TABLE 1 Strains used in this study

Strain	Relevant genotype	Source, reference, or construction[a]
MG1655	λ^- *rph-1*	*E. coli* Genetic Stock Center no. 7740
JW3832	*rrnB3* Δ*lacZ4787 hsdR514* Δ(*araBAD*)*567* Δ(*rhaBAD*)*568 rph-1* (Δ*dsbA*)::*kan*	3
JW5182	*rrnB3* Δ*lacZ4787 hsdR514* Δ(*araBAD*)*567* Δ(*rhaBAD*)*568 rph-1* (Δ*dsbB*)::*kan*	3
TIM63	MG1655 λ_{att}::(P_{mgrB}-*yfp*)	22
TIM92	MG1655 λ_{att}::(P_{mgrB}-*yfp*) HK_{att}::(P_{tetA}-*cfp*)	22
TIM100	MG1655 Δ*phoQ* λ_{att}::(P_{mgrB}-*yfp*) HK_{att}::(P_{tetA}-*cfp*)	22
TIM148	MG1655 λ_{att}::(P_{phoPQ}-*yfp cat*) HK_{att}::(P_{tetA}-*cfp*)	22
TIM229	MG1655 Δ*phoQ* λ_{att}::(P_{phoPQ}-*yfp cat*) HK_{att}::(P_{tetA}-*cfp*)	22
AML20	TIM92 Δ*mgrB*::FRT	18
AML22	TIM148 (Δ*mgrB*)::*kan*	18
AML41	TIM92 (Δ*dsbA*)::*kan*	P1(JW3832) × TIM92
AML42	TIM100 (Δ*dsbA*)::*kan*	P1(JW3832) × TIM100
AML43	TIM92 (Δ*dsbB*)::*kan*	P1(JW5182) × TIM92
AML44	TIM100 (Δ*dsbB*)::*kan*	P1(JW5182) × TIM100
AML45	TIM92 (Δ*rdoA*)::*kan*	P1(JW3831) × TIM92
AML47	TIM148 (Δ*dsbA*)::*kan*	P1(JW3832) × TIM148
AML48	TIM229 (Δ*dsbA*)::*kan*	P1(JW3832) × TIM229
AML53	TIM148 Δ*mgrB*::FRT	AML22/pCP20
AML54	TIM229 Δ*mgrB*::FRT	18
AML55	TIM148 Δ*mgrB*::FRT (Δ*dsbA*)::*kan*	P1(JW3832) × AML53
AML56	TIM229 Δ*mgrB*::FRT (Δ*dsbA*)::*kan*	P1(JW3832) × AML54
AML65	TIM92 Δ*dsbA*::FRT	AML41/pCP20
AML92	TIM148 (Δ*lacY*)::*aadA*	P1(EAL181) × TIM148
AML93	AML22 (Δ*lacY*)::*aadA*	P1(EAL181) × AML22
AML95	AML55 (Δ*lacY*)::*aadA*	P1(EAL181) × AML55
EAL181	MG1655 (Δ*lacY*)::*aadA*	E. A. Libby and M. Goulian, unpublished data

[a] P1(AAA) × BBB denotes P1 transduction from strain AAA into strain BBB. AAA/pCP20 denotes removal of kanamycin resistance from strain AAA by expression of FLP recombinase via pCP20 and subsequent curing of the plasmid.

tus in the periplasm stimulate PhoQ by modulating the negative feedback loop mediated by MgrB.

MATERIALS AND METHODS

Strain and plasmid construction. Strains used are derivatives of MG1655 (*E. coli* Genetic Stock Center, Yale University) and are listed in Table 1. Plasmids used in this work and the oligonucleotides used to construct plasmids or sequence transposon insertion sites are provided in Table 2 and Table 3, respectively.

Mutations and reporter constructs were transferred between strains by transduction with $P1_{vir}$ (19). Deletions transduced from strains in the Keio Collection (3) were confirmed by PCR using primers that flank the gene. When necessary, kanamycin resistance markers were removed with FLP recombinase by transforming with pCP20 (8) and subsequently curing the plasmid, as described in reference 9.

The plasmids pAL14, pAL15, and pAL16 contain point mutations that generate substitutions of alanine for cysteines C16, C28, and C39 of MgrB, respectively. The plasmids were constructed by overlap-extension PCR, using pAL8 as template and primers P1, P4, P3, and P2 (for pAL14); P1, P6, P5, and P2 (for pAL15); and P1, P8, P7, and P10 (for PAL16). The resulting PCR products were digested with EcoRI and BamHI and ligated to the EcoRI and BamHI sites of pEB52. pAL47 carries *mgrB*(*C28A C39A*) and was constructed by DpnI-mediated site-directed mutagenesis of pAL15 with primers P7 and P8 (26). pAL52 carries *mgrB*(*C16A C28A C39A*) (3C→A) and was constructed by site-directed mutagenesis of pAL47 using primers P3 and P4. pAL54 and pAL59 encode N-terminally

TABLE 2 Plasmids used in this study

Plasmid	Description	Citation
pTrc99a	*lacI*^{q}; P_{trc}-MCS; Amp^r	2
pEB52	pTrc99a with NcoI site removed by cutting with NcoI, blunting with mung bean nuclease, and religation; Amp^r	E. Batchelor and M. Goulian, unpublished data
pAL8	pEB52 P_{trc}-*mgrB*; Amp^r	18
pAL14	pEB52 P_{trc}-*mgrB*(*C16A*); Amp^r	This work
pAL15	pEB52 P_{trc}-*mgrB*(*C28A*); Amp^r	This work
pAL16	pEB52 P_{trc}-*mgrB*(*C39A*); Amp^r	This work
pAL47	pEB52 P_{trc}-*mgrB*(*C28A C39A*); Amp^r	This work
pAL52	pEB52 P_{trc}-*mgrB*(*C16A C28A C39A*) [MgrB(3C→A)]; Amp^r	This work
pAL54	pTrc99a P_{trc}-6×His-*mgrB*; Amp^r	This work
pAL59	pTrc99a P_{trc}-6×His-*mgrB*(*C16A C28A C39A*) [6×HIS-MgrB(3C→A)]; Amp^r	This work
pCP20	*ori*(Ts), FLP recombinase expression plasmid; Amp^r Cm^r	8
pRL27	*ori*R6K, Tn*5*-RL27; Km^r	17

TABLE 3 Primers used in this study

Primer designation	Primer sequence (5′–3′)
P1	GCTAGAATTCTGACATAAGGTAGGTG
P2	AAAGGATCCTCACCACGGGATAAACTGG
P3	GTGGTGTTGGCTGCCTTGCTGCTTTGGGC
P4	GCCCAAAGCAGCAAGGCAGCCAACACCAC
P5	CGCAGGTATTCAACATGATGGCCGATCAGGATGTAC AATTTTTCAGC
P6	GCTGAAAAATTGTACATCCTGATCGGCCATCATGT TGAATACCTGCG
P7	GGATGTACAATTTTTCAGCGGAATTGCTGCCATTA ACCAGTTTATCCC
P8	GGGATAAACTGGTTAATGGCAGCAATTCCGCTGAAA AATTGTACATCC
P9	ATACCATGGGTCACCATCACCACCATCACATGAAA AAGTTTCGATGGGTCGTTCTGGTTGTCGTGG
P10	CATCCGCCAAAACAGCCAAG
P11	GACACAGGAACACTTAACGGC
P12	GAGCATTACGCTGACTTGAC

6×His-tagged (6×HIS) *mgrB* and *mgrB*(*3C→A*) proteins, respectively. The plasmids were constructed by PCR using primers P9 and P2 with templates pAL8 and pAL52, respectively. The resulting PCR products were digested with NcoI and BamHI and ligated into the NcoI and BamHI sites of pTrc99a.

Growth conditions. Liquid cultures were grown at 37°C in minimal A medium (19) supplemented with 0.2% glucose, 0.1% Casamino Acids (Difco), and the indicated concentrations of $MgSO_4$. The *trc* promoter was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) at the concentrations indicated. When IPTG is not mentioned in the description of culture conditions, basal transcription from the *trc* promoter was used to drive expression.

Fluorescence images of agar plates. Strains were grown overnight on LB Miller agar (Difco) plates at 37°C. Yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) fluorescence images were acquired with a fluorescence illuminator, as described previously (27). Images were adjusted for brightness and contrast using ImageJ (National Institutes of Health; http://rsbweb.nih.gov/ij/).

Transposon mutagenesis screen. Insertional mutagenesis was performed on the *mgrB* transcriptional reporter strain TIM63 by using the Tn*5*-RL27 transposon derivative pRL27 (17), whose transposable element bears an *ori*R6k and a kanamycin cassette. Mutants that appeared to have increased YFP expression, as determined by fluorescence images of colonies on LB agar plates supplemented with 25 μg/ml kanamycin, were transduced into the P_{mgrB}-*yfp* transcriptional reporter strain TIM92, which also constitutively expresses CFP, as an internal control. Transductants were selected on medium containing 35 μg/ml kanamycin. If mutants retained increased YFP expression, plasmids containing transposon insertions were recovered as described in reference 5, except that the DNA was sheared by sonication. Plasmid DNA was sequenced using primers P11 and P12 to identify the location and orientation of the transposon insertion.

Fluorescence measurements. Cellular fluorescence was measured by microscopy, essentially as described previously (18). Briefly, overnight cultures grown in minimal A medium with 1 mM $MgSO_4$, and with 50 μg/ml ampicillin to maintain plasmids when necessary, were diluted 1:1,000 into prewarmed (37°C) minimal medium containing 100 μM, 1 mM, or 10 mM $MgSO_4$, as indicated. When the reducing agent DTT was used, it was added to the prewarmed medium to a final concentration of 3 mM. Similarly, when the oxidizing agent $CuSO_4$ was used, it was added to a final concentration of 50 μM. Cultures were then grown to an optical density at 600 nm between 0.2 and 0.3 (approximately 4.5 h) and cooled quickly in an ice water slurry, and streptomycin was added to a final concentration of 250 μg/ml to inhibit further protein synthesis. Samples were imaged by fluorescence microscopy and analyzed as described in references 21 and 22. For each culture, fluorescence was determined from the mean fluorescence of at least 40 cells.

RESULTS

Screen for negative regulators of the PhoQ/PhoP system. We screened for negative regulators of the PhoQ/PhoP two-component system by using transposon mutagenesis. The host strain contained the PhoP-regulated *mgrB* promoter driving YFP expression (P_{mgrB}-*yfp*) integrated at the phage lambda attachment site. Growth on LB is a moderately activating condition for the PhoQ/PhoP system (16), likely due to low concentrations of Mg^{2+} and Ca^{2+} in this growth medium (24). Therefore, for cells grown on LB, inactivation of a gene encoding a negative regulator should produce an increase in P_{mgrB}-*yfp* transcription (18). From approximately 12,000 kanamycin-resistant colonies, we identified 10 colonies that displayed increased YFP fluorescence on LB agar plates. We determined the insertion site for six of these mutants. Four proved to be independent insertions in *dsbA*; one was in the 3′ end of *rdoA*, a gene upstream of and in an operon with *dsbA* (6), and one was located between the promoter and start codon of *mgrB*. The remaining four mutants could be complemented with a plasmid expressing DsbA and therefore were not analyzed further. Deletion of *rdoA* (replacement with a kanamycin resistance gene by P1 transduction from a strain in the Keio collection [3]) had no effect on YFP fluorescence, whereas a similar deletion of *dsbA* resulted in a large increase in fluorescence (Fig. 1A). Deletion of *dsbA* in a strain containing a reporter for a different PhoP-regulated promoter (P_{phoPQ}-*yfp*) similarly showed an increase in fluorescence relative to the *dsbA*$^+$ strain under both activating (100 μM) and repressing (10 mM) levels of Mg^{2+} (Fig. 1B). We also noted that deletion of *dsbA* in a Δ*phoQ* strain did not increase P_{mgrB}-*yfp* or P_{phoPQ}-*yfp* transcription (Fig. 1A and B). That deletion of *dsbA* leads to a PhoQ-dependent increase in expression for at least two PhoP-regulated genes suggests that DsbA may act at an upstream pathway common to the entire PhoP regulon.

Conditions that affect disulfide bonding in the periplasm regulate the activity of PhoQ/PhoP. DsbA requires the oxidizing activity of the transmembrane protein DsbB for recycling reduced DsbA to its active form (13, 15, 23). We therefore tested the effect of deleting *dsbB* in a P_{mgrB}-*yfp* reporter strain and observed a *phoQ*-dependent increase in *mgrB* transcription that was similar to the behavior of a *dsbA* deletion (Fig. 1A). We also found that addition of the reducing agent DTT increased YFP fluorescence for P_{mgrB}-*yfp* (Fig. 2A) and P_{phoPQ}-*yfp* (Fig. 2B), which was similar to the behavior of *dsbA* null strains that were not treated with a reducing agent. These results are consistent with a requirement for periplasmic disulfide bond formation in a pathway that represses transcription of PhoP-regulated genes.

The redox active metal ion Cu^{2+} rescues defects in periplasmic disulfide bond formation in *dsbA* null strains (12). To determine whether copper could similarly suppress the increased PhoP-regulated transcription associated with a *dsbA* deletion, we grew Δ*dsbA* strains in the presence of $CuSO_4$ and measured the effect on YFP fluorescence. Addition of as little as 50 μM $CuSO_4$ resulted in levels of transcription of *phoPQ* (Fig. 3) and *mgrB* (data not shown) that were indistinguishable from the corresponding levels in a *dsbA*$^+$ strain grown in the absence of $CuSO_4$, suggesting that

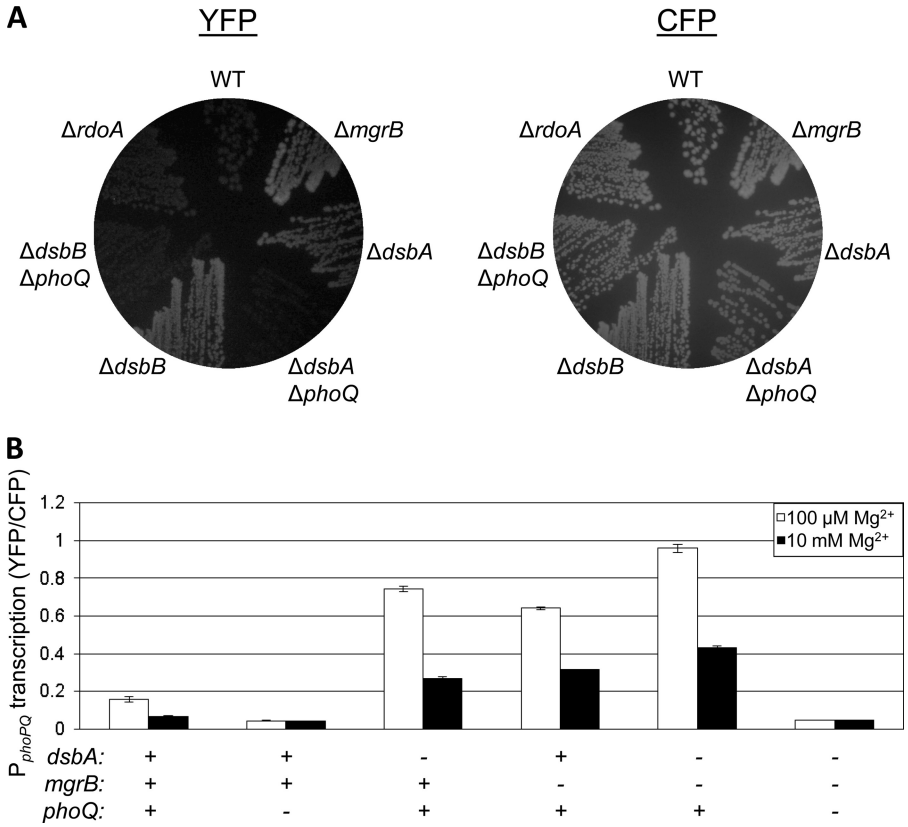

FIG 1 Deletion of *dsbA* increases transcription of *mgrB* and the *phoPQ* operon. The strains contained a chromosomal copy of the *mgrB* promoter (P_{mgrB}) (A) or the autoregulated *phoPQ* promoter (P_{phoPQ}) (B) driving *yfp* expression and a constitutive promoter driving *cfp* expression as an internal reference. (A) YFP (left) and CFP (right) fluorescence images of a transcriptional reporter strain with the indicated gene deletions, grown on LB agar. The strains are, clockwise starting with the wild type (WT), TIM92, AML20, AML41, AML42, AML43, AML44, and AML45. (B) Cells were grown in minimal glucose medium with 100 μM or 10 mM $MgSO_4$. For each condition, the means and ranges for two independent cultures are shown. The strains are, from left to right, TIM148, TIM229, AML47, AML53, AML55, and AML56.

the increased transcription of the PhoP regulon results from a deficiency in periplasmic disulfide bonding.

The inhibitory effect of DsbA on PhoP-regulated transcription is mediated by MgrB. The periplasmic domain of *E. coli* PhoQ does not have any cysteines. However, MgrB, a small 47-amino-acid membrane protein that negatively regulates PhoQ (18), contains three conserved cysteines (C16, C28, and C39) (Fig. 4A). C16 is predicted to be in the transmembrane domain, and C28 and C39 are predicted to be in the periplasm (18). In a P_{phoPQ}-*yfp* reporter strain, deletion of both *mgrB* and *dsbA* resulted in only a slight increase in YFP fluorescence compared with the fluorescence levels in strains containing either deletion alone (Fig. 1B). This suggests that DsbA repression of PhoQ/PhoP signaling may act through the same pathway as that of *mgrB*.

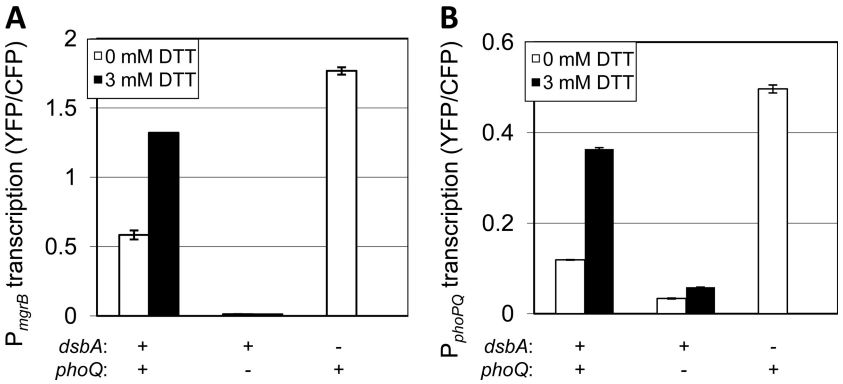

FIG 2 The reducing agent DTT increases transcription of *mgrB* and the *phoPQ* operon. Reporter strains for transcription of the PhoP-regulated genes *mgrB* (A) and *phoPQ* (B) were grown in minimal glucose medium with 1 mM $MgSO_4$ with no added DTT or 3 mM DTT. For each condition, the means and ranges for two independent cultures are shown. Strains are, from left to right, TIM92, TIM100, and AML65 (A) and TIM148, TIM229, and AML47 (B).

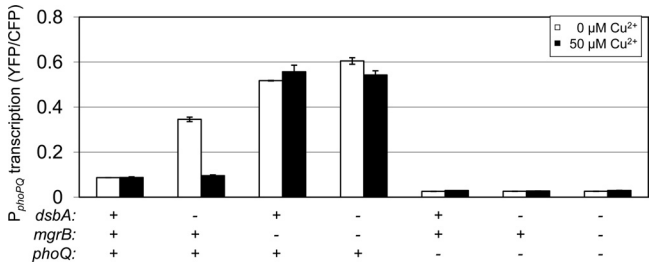

FIG 3 Copper rescues the transcriptional phenotype of a *dsbA* deletion, but not an *mgrB* deletion. Reporter strains for *phoPQ* transcription were grown in minimal glucose medium with 1 mM $MgSO_4$ and either no added $CuSO_4$ or 50 μM $CuSO_4$. For each condition, the means and ranges for two independent cultures are shown. Strains are, from left to right, TIM148, AML47, AML22, AML55, TIM229, AML48, and AML56.

To explore the role of the cysteines in MgrB, we tested the effects of cysteine-to-alanine substitutions. MgrB, expressed from a plasmid at basal levels from the uninduced *trc* promoter, complemented a chromosomal deletion of *mgrB*, as determined by repression of PhoP-regulated transcription (18) (Fig. 4B). Substitution of C16 for an alanine in MgrB [MgrB(C16A)] similarly repressed reporter gene expression. MgrB(C28A), MgrB(C39A), MgrB(C28A C39A), and MgrB with all three cysteines replaced with alanine [MgrB(3C→A)], on the other hand, did not result in repression (Fig. 4B). However, when the *trc* promoter was induced with IPTG, these four MgrB C-to-A mutants showed significant repression (data not shown). We took advantage of this fact to determine whether DsbA exerts its effect on PhoP-regulated transcription through the cysteines of MgrB. We tested the effect of deletion of *dsbA* in a *phoPQ* reporter strain that lacked *mgrB* and contained either a plasmid expressing MgrB from the *trc* promoter without inducer, a plasmid expressing MgrB(3C→A) induced from the *trc* promoter with 68 μM IPTG, or an empty control plasmid. The inducing condition of 68 μM IPTG for MgrB(3C→A) was chosen because it was determined to give comparable levels of repression to that of (wild-type) MgrB without inducer. As expected, deletion of *dsbA* led to increased reporter transcription in the strain expressing MgrB (Fig. 4C). However, deletion of *dsbA* had no effect for the strain expressing MgrB(3C→A). This suggests that DsbA exerts its effect on the PhoQ/PhoP system by acting on MgrB or in a pathway upstream

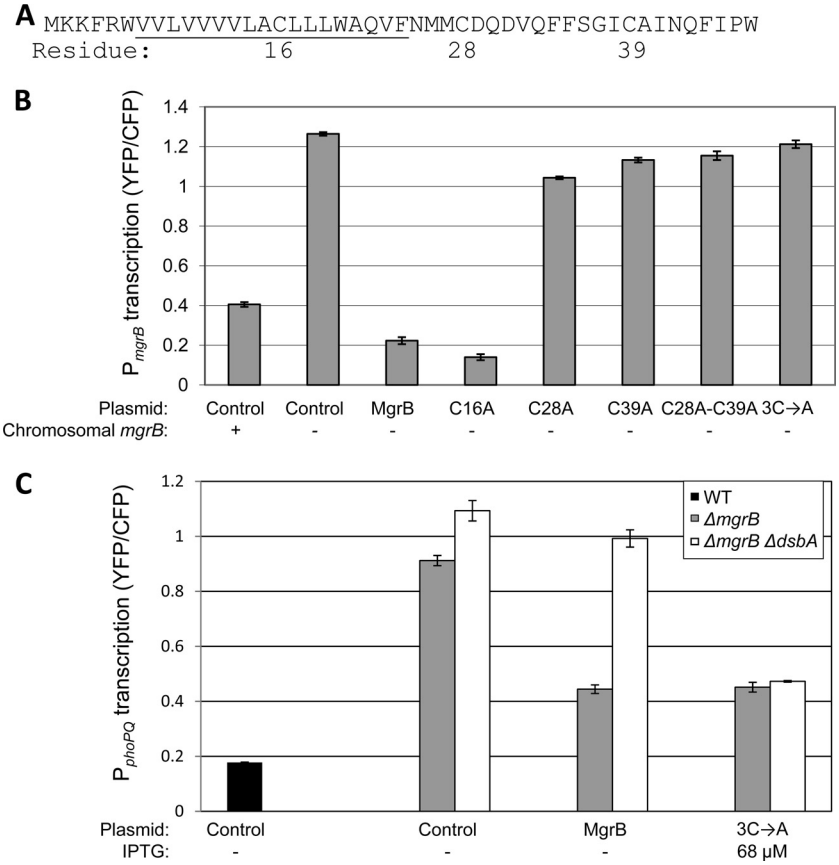

FIG 4 DsbA affects PhoQ/PhoP signaling through the cysteine residues of MgrB. (A) Amino acid sequence of MgrB. The underlined portion indicates the predicted transmembrane domain. (B) Reporter strains for *mgrB* transcription are either wild type for chromosomal *mgrB* (TIM92) or carry a deletion (AML20) and contain either a control plasmid (pEB52), a plasmid expressing MgrB (pAL8), or a plasmid expressing an MgrB mutant with the following amino acid substitutions: C16A (pAL14), C28A (pAL15), C39A (pAL16), C28A and C39A (pAL47), or C16, C28A, and C39A (3C→A; pAL52). (C) Reporter strains for *phoPQ* operon transcription with either wild-type chromosomal *mgrB* (AML92), a deletion of *mgrB* (AML93), or a deletion of both *mgrB* and *dsbA* (AML95) were transformed with a control plasmid (pTrc99a), a plasmid expressing 6×HIS-MgrB (pAL54), or a plasmid expressing 6×HIS-MgrB(3C→A) (pAL59). For each condition, the means and ranges for two independent cultures are shown. Cells were grown in minimal glucose medium with 100 μM $MgSO_4$ and without IPTG, except for the strain harboring the 6×HIS-MgrB(3C→A) plasmid, which was grown in medium with 68 μM IPTG.

of MgrB. Consistent with this interpretation, we also found that Cu^{2+} failed to decrease reporter gene expression in a Δ*mgrB* strain as well as in a Δ*dsbA* Δ*mgrB* double mutant (Fig. 3).

DISCUSSION

The results presented here indicate that the redox state of the periplasm plays a critical role in the regulation of the *E. coli* PhoQ/PhoP circuit, with reducing conditions resulting in stimulation of this two-component system. Null mutations in *dsbA* or *dsbB*, or treatment with DTT, increased expression of PhoP-regulated genes in a PhoQ-dependent manner, while treatment with the oxidant Cu^{2+} suppressed the effects of a *dsbA* deletion. Taken together, these results suggest that disruption of one or more disulfide bonds in the periplasm stimulates the PhoQ/PhoP system.

We have also provided evidence that this redox sensitivity is mediated by the small membrane protein MgrB. In particular, expression of an MgrB mutant in which the three cysteines were replaced with alanines completely blocked the effect of a *dsbA* null mutation on PhoQ/PhoP signaling (Fig. 4C). There was, however, a small increase in PhoP-regulated transcription in a Δ*dsbA* Δ*mgrB* strain compared with a strain that had a deletion of only one of the two genes (Fig. 1B). This may indicate an additional effect of DsbA on PhoQ/PhoP signaling under conditions in which PhoP-P levels are in excess (due to the absence of MgrB). Our results are consistent with disulfide bonding by one or more MgrB cysteines playing a role in the repressive effect of MgrB on PhoQ. In addition, the behavior of specific MgrB cysteine-to-alanine mutants on PhoP-regulated transcription is consistent with the two cysteines in the periplasm (C28 and C39) forming either inter- or intramolecular disulfide bonds. The activity of MgrB mutants with C28A and/or C39A substitutions could be restored by increased expression of the mutants from an inducible promoter. This indicates that C28 and C39 are not absolutely required for MgrB to repress PhoQ, although these cysteine residues may be important for stabilizing a protein conformation or complex that is critical for MgrB's repressive action. Taken together, the results suggest a model in which DsbA affects PhoQ/PhoP signaling by oxidizing C28 and C39 of MgrB. At present, however, we cannot rule out alternative models in which these cysteines are in a reduced state and DsbA does not directly interact with MgrB. Further work will be required to establish the disulfide bonding status of the MgrB cysteines and to determine whether disulfide bonding of other proteins participates in this DsbA pathway.

We do not know whether *E. coli* encounters in its native environments conditions that are similar to the reducing conditions studied here. However, it is noteworthy that the oxidation potential of extracellular fluids in animals is quite variable and depends on numerous factors, including age, disease state and, for the gastrointestinal tract, diet (11). In addition, antimicrobial defenses may interfere with disulfide bond formation in the *E. coli* envelope through the production of reactive compounds that modify free sulfhydryls or disrupt disulfide bonding pathways. It is therefore possible that changes in periplasmic redox conditions or a related property of the cell envelope may be an important input for the sensor kinase PhoQ and may contribute to the ability of *E. coli* to adapt to environments in animal hosts.

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