

# The Activity and Specificity of the Outer Membrane Protein Chaperone SurA Are Modulated by a Proline Isomerase Domain

Dante P. Ricci,\* Jaclyn Schwalm, Michelle Gonzales-Cope, Thomas J. Silhavy

Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA

\* Present address: Department of Developmental Biology, Stanford University School of Medicine, Stanford, California, USA

**ABSTRACT** SurA is a component of the periplasmic chaperone network that plays a central role in biogenesis of integral outer membrane  $\beta$ -barrel proteins (OMPs) in *Escherichia coli*. Although SurA contains two well-conserved proline isomerase (PPIase) domains, the contribution of these domains to SurA function is unclear. In the present work, we show that defects in OMP assembly caused by mutation of the  $\beta$ -barrel assembly factors BamA or BamB can be corrected by gain-of-function mutations in SurA that map to the first PPIase domain. These mutations apparently bypass the requirement for a stable interaction between SurA and the Bam complex and enhance SurA chaperone activity *in vivo* despite destabilization of the protein *in vitro*. Our findings suggest an autoinhibitory mechanism for regulation of SurA chaperone activity through interdomain interactions involving a PPIase domain. We propose a model in which SurA activity is modulated by an interaction between SurA and the Bam complex that alters the substrate specificity of the chaperone.

**IMPORTANCE** The dominant *surA* mutations described here alter amino acid residues that are highly conserved in eukaryotic homologs of SurA, including Pin1, the human proline isomerase (PPIase) implicated in Alzheimer's disease and certain cancers. Consequently, a mechanistic description of SurA function may enhance our understanding of clinically important PPIases and their role(s) in disease. In addition, the virulence of Gram-negative bacterial pathogens, such as *Salmonella*, *Shigella*, and *Escherichia coli* O157:H7, is largely dependent on SurA, making this PPIase/chaperone an attractive antibiotic target. Investigating the function of SurA in outer membrane (OM) biogenesis will be useful in the development of novel therapeutic strategies for the disruption of the OM or the processes that are essential for its assembly.

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Address correspondence to Thomas J. Silhavy, [tsilhavy@princeton.edu](mailto:tsilhavy@princeton.edu).

The outer membrane (OM) of Gram-negative bacteria, which primarily serves as a robust diffusion barrier, is distinguishable from the inner membrane (IM) in its relative asymmetry and unique constitution. Specifically, the OM harbors a distinct species of integral membrane protein that adopts a  $\beta$ -barrel fold and is specific to this extracytoplasmic compartment. These integral OM proteins (OMPs) are highly insoluble, aggregation-prone polypeptides that achieve native structure only in the cell membrane; consequently, molecular chaperones are required to maintain nascent OMPs in a folding-competent state as they exit the IM translocase (Sec) and traverse the aqueous periplasmic compartment in transit to the OM-localized OMP assembly complex known as Bam (1).

The periplasmic protein SurA has long been known to participate in assembly of integral outer membrane  $\beta$ -barrel proteins (2–4). The significant reduction in the levels of folded OMPs and the accumulation of unfolded periplasmic OMP species observed upon disruption of *surA* (4) provided early evidence for the contribution of SurA to OMP biogenesis. SurA plays a vital role in the maintenance of OM integrity in *Escherichia coli*, as *surA* mutants possess a defective OM that leaks periplasmic contents and per-

mits the influx of detergents and toxic small molecules (2–4). SurA has been regarded as a periplasmic OMP chaperone since the time of its discovery, but a clear understanding of the mechanism by which SurA promotes OMP biogenesis is lacking (5).

Although the folding and assembly of many OMPs are impacted by deletion of *surA* (2, 4), only a small number of OMPs are strongly SurA dependent; a notable example is LptD, the essential lipopolysaccharide (LPS) insertase (6). The viability of *surA* mutants reflects the existence of an auxiliary OMP transport pathway comprising the chaperone Skp and the protease/chaperone DegP (7, 8). Yet, given the indirect but critical role for SurA in LPS assembly, the accumulation of misassembled OMPs observed in its absence, and the fact that disruption of *surA* is poorly tolerated (especially in combination with other mutations affecting OM biogenesis) (8–11), SurA is considered to be the primary OMP chaperone in the *E. coli* periplasm (6, 7, 12).

The architecture of SurA raises interesting questions about its activity. Structural analysis of SurA (13, 14) revealed a core module, which is formed from the N and C termini, and two proline isomerase (PPIase) domains of the parvulin type (referred to here as P1 and P2). The P1 domain associates extensively with the core

module, whereas the peripheral P2 domain is tethered to the core by an extended linker. *In vivo* and *in vitro* analysis of SurA derivatives lacking one or more of the various domains described above led to the conclusion that the chaperone activity of SurA is contained within the core module; deletion of one or both parvulin domains has little impact on SurA chaperone activity, and the core module alone is largely sufficient to complement the function(s) of SurA *in vivo* (15). Only the second PPIase domain (P2) actually exhibits significant PPIase activity (4, 15), and this activity seems to be dispensable for SurA function (15). However, the strong conservation of PPIase domains among SurA homologs and the presence of multiple proline isomerases in the periplasm suggest that these domains of SurA have some physiological relevance (16).

SurA interacts with the periplasmic domain of the central OMP assembly factor, BamA, in a manner that can be stabilized by cross-linking agents (7, 17), and formation of this complex does not require the accessory lipoprotein BamB (18). The fact that deletion of BamB has no effect on the binding of SurA to Bam is somewhat surprising, as SurA and BamB have been proposed to function in the same folding pathway for several OMPs (6, 18–21). The molecular determinants of the interaction between SurA and BamA are not yet clear, although recent evidence suggests that the N-terminal POTRA domain of BamA (POTRA 1) is involved in this physical interaction (17, 22). Although the BamA periplasmic domain likely influences various aspects of the OMP assembly reaction, the importance of the POTRA domains must, at least in part, reflect their role in chaperone docking and substrate transfer.

We report here the isolation of a BamA mutant that impacts the functional and physical connection between SurA and the Bam complex. We show that the OMP misassembly that occurs upon disruption of the BamB pathway can be reversed by compensatory mutations within *surA*. Strikingly, these *surA* mutations are found within the first parvulin domain of SurA (P1). This finding represents the first direct evidence that implicates a PPIase domain of SurA in OMP biogenesis. Furthermore, the identification of these parvulin domain mutations in SurA provides compelling genetic evidence for the existence of two separable SurA-dependent OMP assembly pathways: one that involves BamB, and one that does not. The mutants reported here inform an integrated model for OMP targeting in the periplasm that offers insight into the role of Bam and a proline isomerase domain in the regulation of SurA activity.

## RESULTS

**Bam function is compromised in a *bamA616* background.** To generate a library of partial loss-of-function *bamA* mutants, we conducted a screen for *bamA* mutations that decrease resistance to the detergent SDS, a phenotype that indicates impaired OM biogenesis (23). We describe here *bamA616*, a detergent-hypersensitive mutant isolated in this screen. This allele was found to encode three missense mutations (R91H, R162L, Y317H), but through construction of single and double mutants of every combination, we determined that the R91H and R162L mutations together are necessary and sufficient to cause the *bamA616* phenotype. The pertinent substitutions that define *bamA616* involve two residues lying at the interface between the first and second POTRA domains of BamA (see Fig. S1 in the supplemental material).

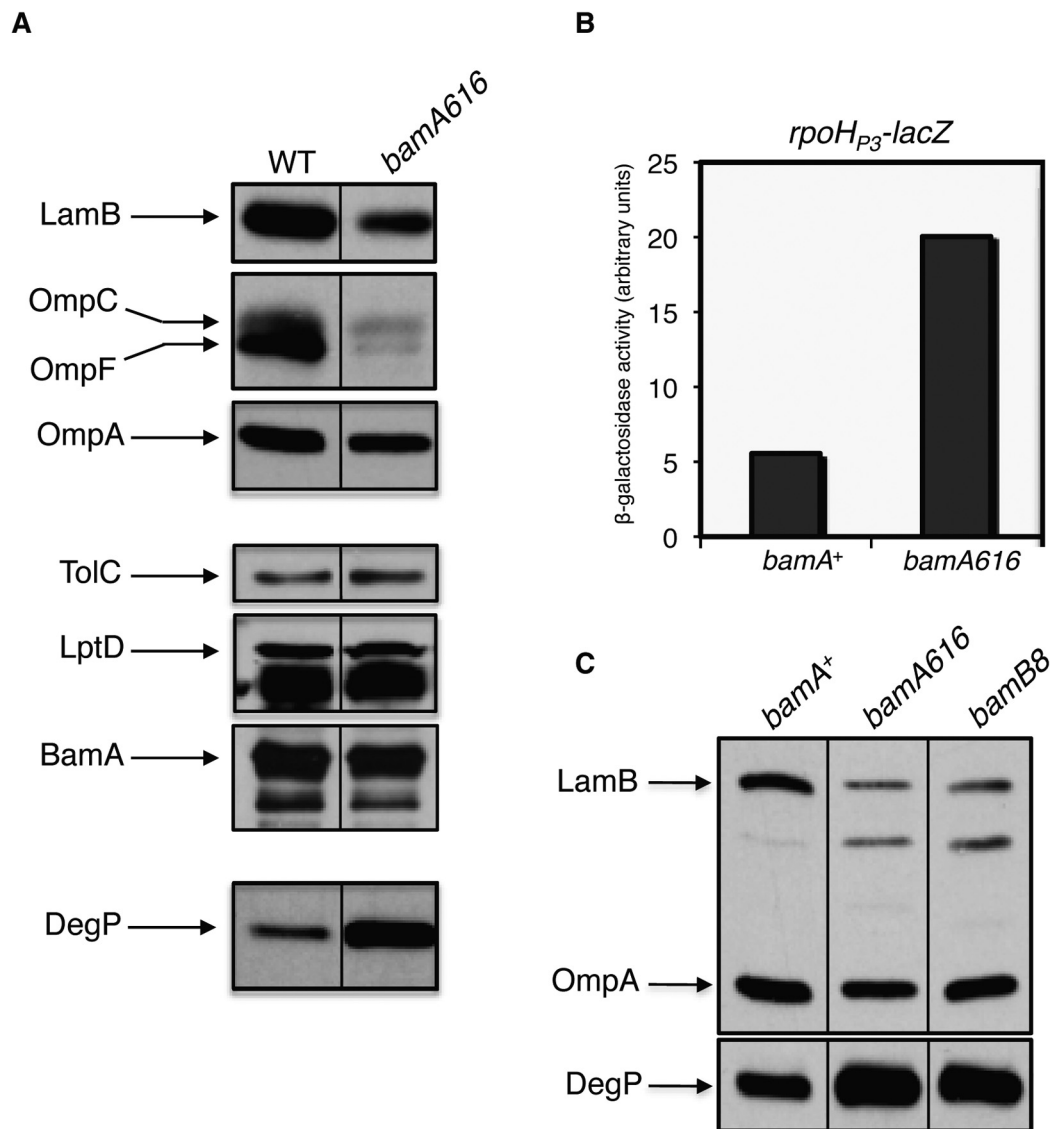
In addition to SDS, *bamA616* strains exhibit increased susceptibility to antibiotic compounds with various physicochemical

properties (see Table S1a in the supplemental material). To determine whether this increased drug sensitivity reflects a defect in OMP assembly, we determined the steady-state levels of various OMPs in this mutant strain. We observed reduced levels of abundant OMPs (particularly LamB and OmpF) in this background (Fig. 1a), an effect attributable to misassembly, degradation, and reduced synthesis of major OMPs (6, 24, 25). In addition, we observed a marked increase in the levels of the periplasmic protease DegP (Fig. 1a) and a 4-fold increase in expression from the *rpoH<sub>P3</sub>* promoter (Fig. 1b); both of these findings indicate activation of the  $\sigma^E$  extracytoplasmic stress response through a signaling cascade induced by unfolded periplasmic OMPs (26). Together, these findings indicate that OMP biogenesis is impacted by the *bamA616* mutations. This effect is limited to a subset of OMPs, as the levels of LptD, BamA, and TolC are unchanged in the mutant background (Fig. 1a).

We note that *bamA616* strains bear a striking phenotypic resemblance to those that carry *bamB8*, a previously characterized null mutation that abrogates the function of the accessory lipoprotein BamB (9). *bamA616* and *bamB8* have similar antibiotic and small molecule sensitivity profiles, and the levels of affected OMPs are similarly reduced in each mutant background (Fig. 1c). In addition, rather than observing a global decrease in OMP levels in *bamA616* and *bamB8* strains (as occurs upon BamA or BamD depletion), each mutation results in a decrease in levels of major OMPs (such as LamB and OmpF) but not LptD or BamA (6). Finally, we are unable to construct a strain containing both *bamA616* and *imp4213* by P1 transduction (data not shown); this is striking considering the reported synthetic lethality of *bamB8 imp4213* double mutants (23, 27). While *bamA616* strains seem to exhibit a *bamB*-null-like phenotype, we do not observe an obvious change in binding of BamB to BamA<sup>616</sup> compared with that of BamA (see Text in the supplemental material and Fig. 2), suggesting that the *bamA616* phenotype is not simply caused by the physical dissociation of BamA and BamB.

**Binding of SurA to BamA is impaired in a *bamA616* background.** Several lines of evidence suggest that some OM  $\beta$ -barrels are targeted to and assembled by BamA via a folding pathway that includes both BamB and the periplasmic chaperone SurA (6, 18–20). Given the *bamB*-null-like phenotype of the *bamA616* mutant and the apparent functional relationship between SurA and BamB, we considered the possibility that SurA function is somehow compromised in a *bamA616* background.

The Bam proteins form a stable complex that is readily purified intact, and no other OMPs or envelope proteins are known to stably associate with Bam in *E. coli* (see Fig. S2 in the supplemental material) (28, 29). However, SurA is known to interact with the Bam complex via a physical interaction that can be trapped with chemical cross-linking agents (7, 17, 18). As such, we first sought to determine whether the *bamA616* mutations influences this interaction. Copurification experiments using wild-type His-Bam (His-BamA<sup>WT</sup>) or His-BamA<sup>616</sup> as described above were repeated in the presence of the OM-permeable cross-linking agent DSP [dithiobis(succinimidyl propionate)]. Upon purification, we observe that both His-BamA<sup>WT</sup> and His-BamA<sup>616</sup> form detectable interactions with SurA. However, there is an apparent 50% decrease in the amount of SurA that copurifies with His-BamA<sup>616</sup> compared to that with His-BamA<sup>WT</sup> (Fig. 2, top). This decrease suggests that the physical interaction between BamA and SurA is impacted by the *bamA616* mutations.

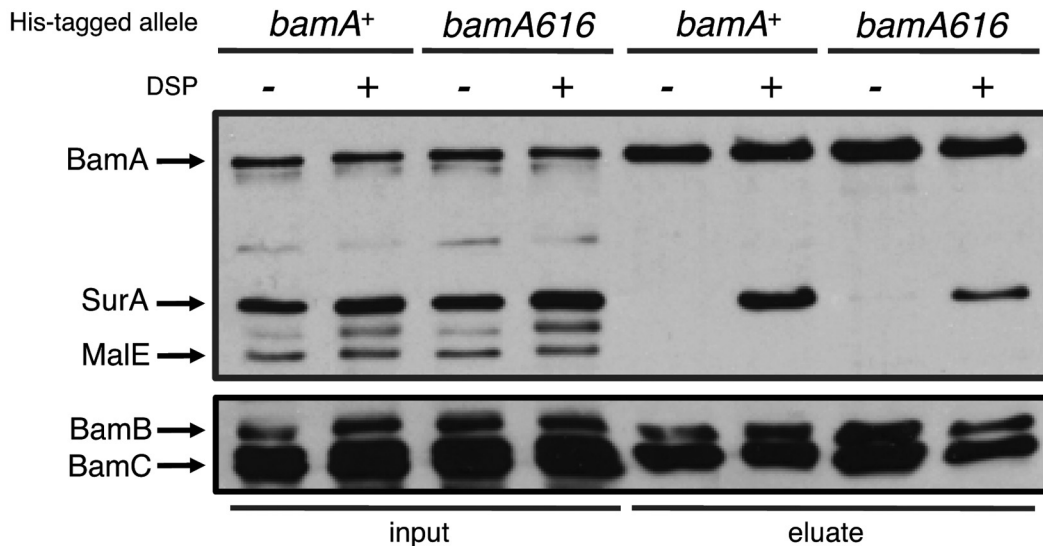


**FIG 1** *bamA616* impacts biogenesis of major OMPs. (A) Relative OMP levels were determined in whole-cell extracts of *bamA*<sup>+</sup> and *bamA616* strains that were subjected to SDS-PAGE and immunoblotting for the proteins indicated. (B)  $\sigma^E$  activity was assayed by measuring  $\beta$ -galactosidase activity in *bamA*<sup>+</sup> and *bamA616* strains containing a chromosomal copy of the  $\sigma^E$ -dependent *rpoH*<sub>P<sub>3</sub></sub>-*lacZ* reporter fusion. (C) Whole-cell extracts of *bamA*<sup>+</sup>, *bamA616*, and *bamB8* strains were prepared, and envelope proteins were detected as described for panel A.

**Mutations in *surA* suppress *bamA616*.** In order to better understand the physical and functional interaction(s) between SurA, BamB, and the N-terminal POTRA domains of BamA, we exploited the detergent sensitivity of *bamA616* mutant to select for mutations that reverse the OM biogenesis defects observed in a *bamA616* background. By selecting spontaneous SDS-EDTA<sup>r</sup> revertants at 37°C, we identified two extragenic suppressor mutations that confer wild-type SDS-EDTA resistance to a *bamA616* strain but differ in their effect on colony size: one suppressor strain forms normal colonies on rich media, whereas the other forms abnormally small colonies. Despite the difference in colony morphology, we found that both of these suppressor strains carry mutations in the *surA* gene. We refer to the suppressor alleles here as *surA10* and *surA13*, which give rise to normal and small colonies, respectively, in a *bamA616* background. The *surA10* mutation re-

sults in a Ser-to-Ala substitution in SurA at residue 220 (SurA<sup>S220A</sup>). *surA13* encodes a deletion of Leu215 and Ala216 (SurA<sup>ΔLA</sup>).

In addition to restoring SDS-EDTA resistance, both *surA* mutations reduce the sensitivity of a *bamA616* strain to other antibiotics, albeit to different degrees (see Table S1a in the supplemental material). The increase in resistance to multiple antibiotic compounds shows that these suppressors are not acting to restrict the influx of SDS exclusively. This suggests that the *surA10* and *surA13* mutations influence the composition of the OM, perhaps by correcting the defect in OMP maturation that is observed in a *bamA616* background. To test this, we measured steady-state OMP levels and quantified  $\sigma^E$  stress response activation in the suppressor strains. In the *bamA616 surA10* double mutant, we observe a substantial increase in the steady-state levels of LamB,



**FIG 2** The interaction between SurA and BamA is compromised by *bamA616*. DSP cross-linking and affinity purification were performed in cells expressing His<sub>6</sub>-tagged BamA<sup>+</sup> (lanes 1, 2, 5, and 6) or BamA<sup>616</sup> (lanes 3, 4, 7, and 8). Cells were incubated in the presence of DSP (even lanes) or DMSO (odd lanes) prior to lysis. Whole-cell extracts (lanes 1 to 4) and Ni-nitrilotriacetic acid (NTA)-enriched eluate fractions (lanes 5 to 8) were subjected to SDS-PAGE and immunoblotting for the indicated envelope proteins.

OmpF, and OmpA, such that the cellular amounts of these OMPs approximated the levels observed in wild-type cells (Fig. 3a). In addition, transcriptional activity at the *rpoH*<sub>P<sub>3</sub></sub> promoter (a direct  $\sigma^E$  target) is reduced to baseline levels in the double mutant (Fig. 3b). Taken together, these data show that the *surA10* mutation restores efficient OMP biogenesis in a *bamA616* background.

Surprisingly, LamB levels in the *bamA616 surA13* (small colony) suppressor strain are decreased in comparison to levels in the *bamA616* strain (Fig. 3c). Additionally, we do not observe a significant change in transcriptional activity at the *rpoH*<sub>P<sub>3</sub></sub> promoter compared to that of the *bamA616* single mutant (Fig. 3b). Thus, the *bamA616 surA13* strain, although quite similar to the wild type with respect to OM permeability, has an OMP profile and colony morphology distinct from that of a *bamA616 surA10* strain, its *surA*<sup>+</sup> parent, and the wild type. We have found that this apparent incongruity reflects activation of the Cpx stress response in the *bamA616 surA13* double mutant specifically; because of the pleiotropic effects caused by the *surA13* allele, we here concentrate our analysis on the *surA10* suppressor (see Text and Fig. S3a to c in the supplemental material).

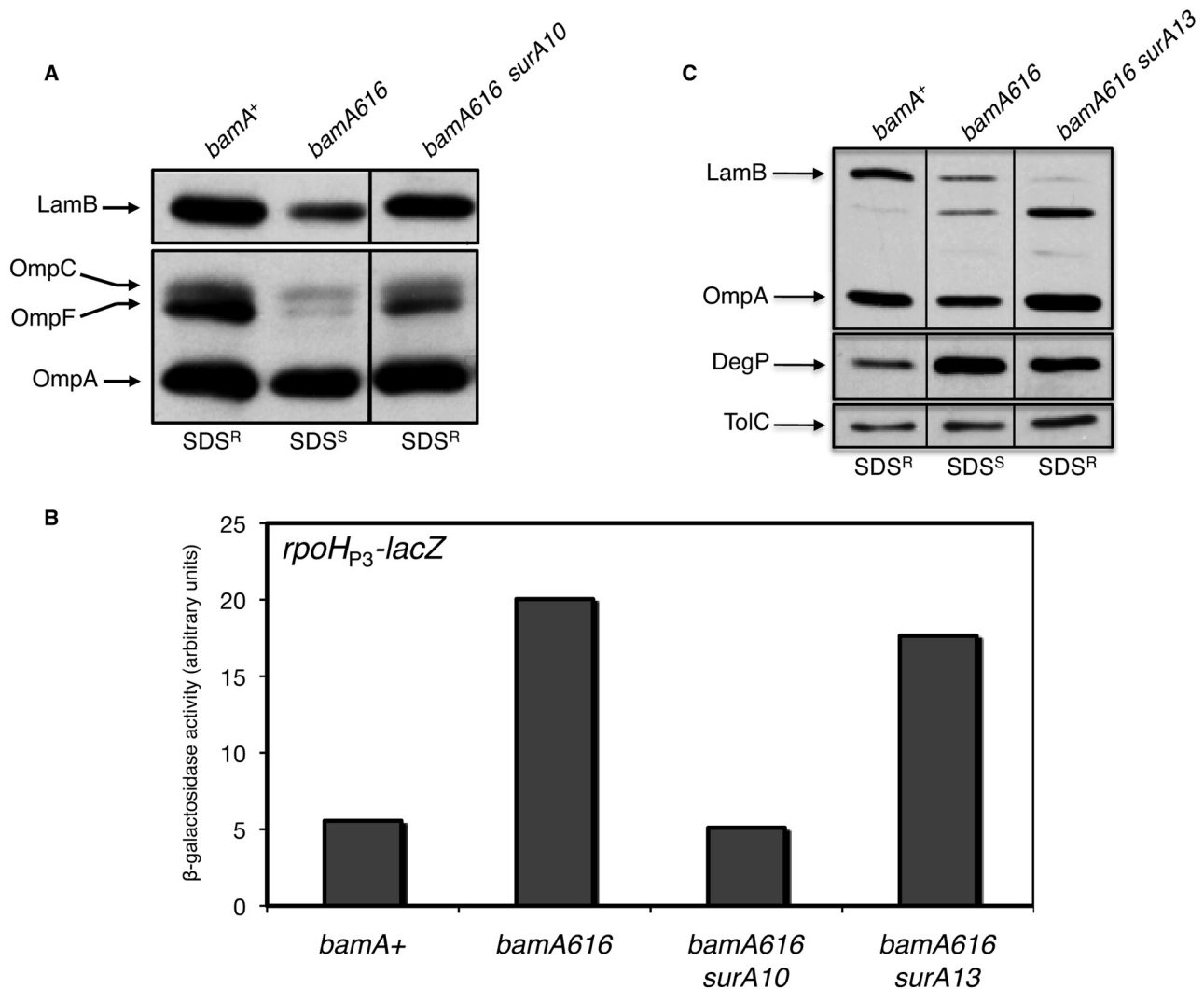
***surA10* does not restore the SurA-BamA<sup>616</sup> interaction.** To determine whether the *surA10* mutation restores binding of SurA to BamA<sup>616</sup>, we performed copurification experiments in *surA10* strains expressing either *bamA*<sup>+</sup> or *bamA616*. We find that SurA<sup>+</sup> and SurA<sup>S220A</sup> can be cross-linked to wild-type BamA in roughly equivalent amounts. However, the amount of SurA<sup>S220A</sup> that copurifies with BamA<sup>616</sup> is even less than is observed for wild-type SurA (Fig. 4). The S220A mutation does not impair the ability of SurA to interact with BamA<sup>+</sup>, nor does it restore the physical interaction between SurA and BamA<sup>616</sup>.

**Mutations in SurA P1 suppress the loss of BamB.** If the phenotypic similarity between the *bamA616* and the *bamB*-null mutants reflects a common defect in an OMP assembly pathway, *surA10* might also be expected to suppress the *bamB*-null mutant. Intriguingly, we found that the *surA10* mutation indeed sup-

presses the detergent sensitivity and OMP assembly defects observed in a *bamB* mutant background (Fig. 5), suggesting that the variant overcomes a defect that arises upon deletion of *bamB* or substitution of BamA residues R91 and R162.

The observation that *surA10* is neither gene specific nor capable of restoring the SurA-BamA<sup>616</sup> interaction is consistent with the notion that *surA10* is a gain-of-function allele. Several additional observations support this hypothesis (see the text in the supplemental material): (i) *surA10* is dominant to *surA*<sup>+</sup> in diploid analysis (see Table S1b in the supplemental material), (ii) *surA10* is sufficient to fully complement  $\Delta$ *surA* (Fig. 6a), (iii) SurA<sup>S220A</sup> exhibits increased activity relative to SurA<sup>+</sup> (Fig. 6b), and (iv) SurA overexpression is not sufficient to suppress *bamA616* (see Table S1b).

**The S220A mutation destabilizes SurA *in vitro*.** All residues affected by the *surA10* and *surA13* mutations (Leu215, Ala216, and Ser220) are highly conserved in both eukaryotic and bacterial parvulins (see Fig. S4a in the supplemental material) and map to the  $\alpha$ 2 helix of the P1 domain; this helix lies along one edge of the antiparallel  $\beta$ -sheet within P1 and forms one side of the peptide binding pocket (see Fig. S4b), although none of the residues described above interact directly with peptide substrates that bind P1 (14). In the full-length SurA protomer, the P1  $\alpha$ 2 helix also forms a major interface between the P1 domain and the core chaperone domain of SurA (see Fig. S4c). We therefore speculated that the *surA10* mutation might influence the stability of the P1-core interaction, potentially impacting global stability of the protein. In order to test this, we released periplasmic proteins from cells expressing *surA*<sup>+</sup> or *surA10* by chloroform shock and determined the fate of SurA and SurA<sup>S220A</sup> following release. We found that SurA<sup>+</sup> can be observed in protein extracts following incubation at 37°C. However, SurA<sup>S220A</sup> is undetectable under identical conditions, suggesting that the protein is destabilized and degraded after periplasmic release (Fig. 6c). As a control, we show that the



**FIG 3** The *surA10* and *surA13* suppressors restore detergent resistance but differentially influence OMP levels. (A) Whole-cell extracts of strains indicated were prepared by boiling after harvesting cells at an OD<sub>600</sub> of 1. Major OMPs were detected by SDS-PAGE and immunoblotting. Detergent sensitivity of each strain is shown below the corresponding lane (SDS<sup>R</sup>, detergent resistant; SDS<sup>S</sup>, detergent sensitive). (B)  $\beta$ -Galactosidase activity was measured in the indicated strains containing a transcriptional fusion with the  $\sigma^F$ -dependent *rpoH<sub>P3</sub>* promoter driving expression of *lacZ*. (C) The strains indicated were processed as described for panel A, and the indicated envelope proteins were detected by immunoblotting.

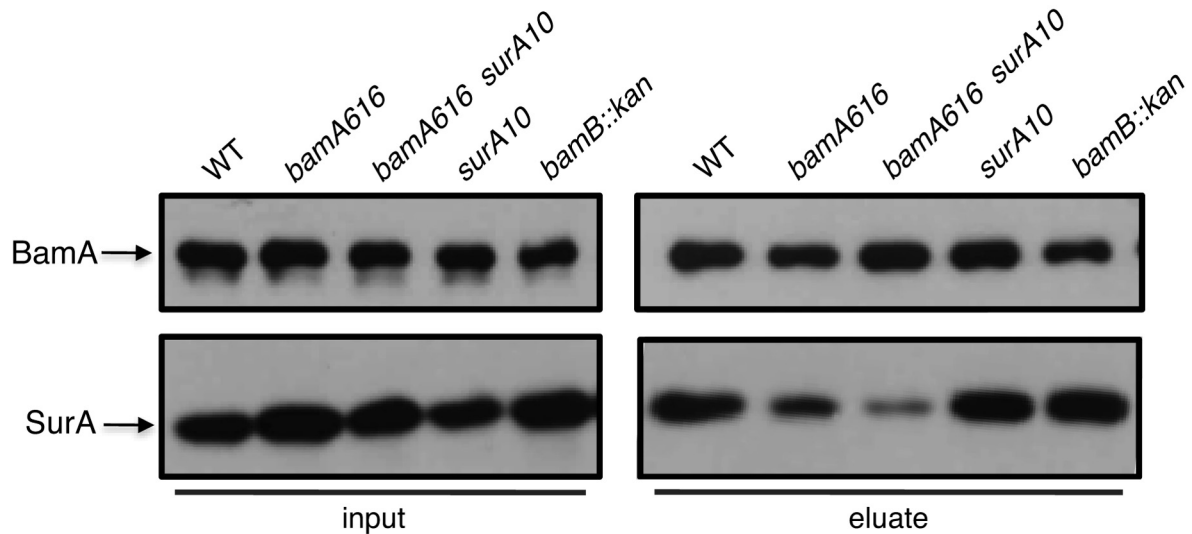
periplasmic protein MalE remains resistant to degradation in either a *surA*<sup>+</sup> or *surA10* background.

To confirm this finding by an independent method, we prepared cell lysates of strains expressing *surA*<sup>+</sup> or *surA10* under conditions in which cellular proteins are solubilized but not denatured. When these strains are lysed gently in a mild detergent solution, brief incubation of cell extracts at room temperature prior to boiling in SDS-PAGE sample buffer leads to the complete degradation of SurA<sup>S220A</sup> but not SurA<sup>+</sup> (see Fig. S5a). Because this degradation can be partially stabilized by the addition of protease inhibitors (data not shown), and because levels of SurA<sup>S220A</sup> in whole cells are comparable to those observed for SurA<sup>+</sup> (Fig. 6a), we interpret this finding to mean that SurA<sup>S220A</sup> is much more susceptible to degradation by cellular proteases following lysis than is SurA<sup>+</sup>.

## DISCUSSION

The mutations that cause the *bamA616* phenotype affect residues at the interface of POTRA 1 and POTRA 2 near the periplasmic N terminus of BamA. POTRA 1 and 2 form a rigid body that is maintained by extensive interdomain contacts (33, 42, 43), and it is possible that this POTRA pair operates as a single module (32). POTRA 1 and 2 have previously been implicated in binding of SurA (17, 22) and BamB (33), and it has been suggested that POTRA 1 is involved in the assembly of a subset of OMPs that includes the archetypal porins OmpF/OmpC and the major OMPs LamB and OmpA (17). Consistent with this hypothesis, we find that *bamA616* specifically affects these abundant OMPs without compromising the assembly of LptD, TolC, or BamA itself.

BamA<sup>616</sup> interacts normally with BamB, so the *bamB*-null-like phenotype of a *bamA616* strain cannot be attributed to the phys-



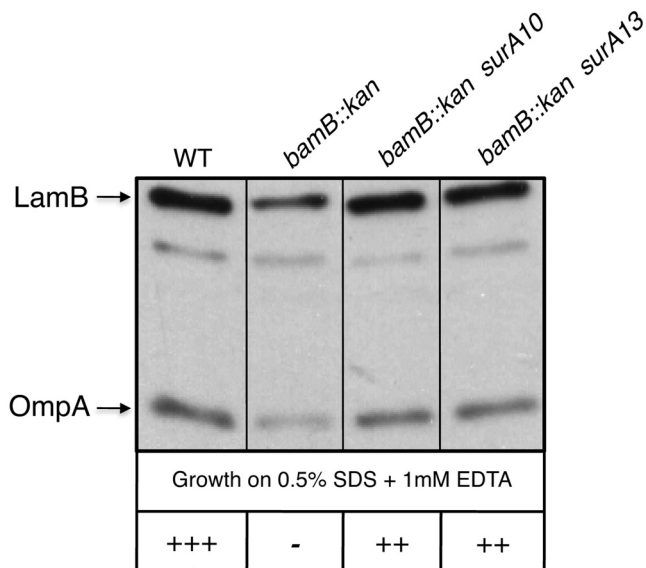
**FIG 4** The *surA10* mutation does not restore the SurA-BamA<sup>616</sup> interaction. DSP cross-linking and affinity purification were performed using His<sub>6</sub>-tagged BamA<sup>+</sup> (lanes 1, 4, and 5) or BamA<sup>616</sup> (lanes 2 and 3). All strains indicated were incubated with the cross-linker DSP prior to lysis. Whole-cell extracts (left) and Ni-NTA-enriched eluate fractions (right) were subjected to SDS-PAGE and immunoblotting for BamA and SurA.

ical dissociation of these two components. We argue instead that POTRA 1/2 and BamB are each involved in a common function that is unmasked in a *bamA616* background or in the absence of BamB. Because binding of SurA to BamA is reduced by the *bamA616* mutations and because both *bamA616* and *bamB* mutant strains exhibit OMP biogenesis defects that are suppressed by mutations in SurA, we conclude that the primary defect in each of these mutant strains likely relates to the activity of SurA (or lack thereof). Our observations implicate the N-terminal POTRA

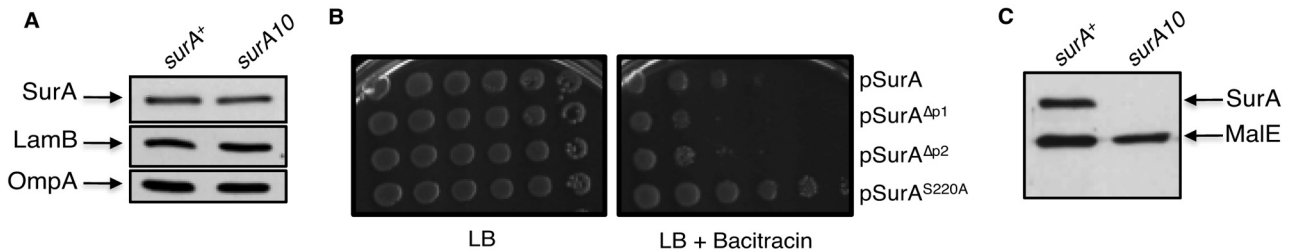
module and BamB in the regulation of SurA function. We suggest that BamAB modulates SurA chaperone activity and that the SurA<sup>S220A</sup> variant mimics the BamAB-induced activity state of SurA.

SurA activity is potentiated by the *surA10* mutation in a manner that cannot be recreated by simply increasing its concentration. Unexpectedly, the S220A (*surA10*) and  $\Delta$ 215-216 (*surA13*) mutations are found not in the chaperone domain of SurA but in the first PPIase domain (P1). *surA10* is a “gain-of-function” mutation in that it increases SurA chaperone activity. Yet, a comparative analysis of parvulins suggests that S220A and  $\Delta$ 215-216 should represent loss-of-function mutations, since substitutions at those highly conserved residues abrogate function in homologs of SurA (34, 35). These observations can be reconciled by proposing that the P1 domain inhibits SurA chaperone activity and that the *surA10* and *surA13* mutations disinhibit the chaperone domain by crippling the inhibitory P1 domain (antirepression). In this way, loss-of-function mutations in an autoinhibitory domain lead to the increased activity of a covalently linked chaperone domain. This model presumes that the activity of full-length SurA is normally at least partially inhibited. Consistent with this proposal, the *in vitro* chaperone activity of SurA is significantly increased upon the deletion of one or both parvulin domains (15). Since the parvulin domains have no intrinsic chaperone activity, they likely act by modulating the activity of the chaperone domain.

Our model further implies that the relief of this autoinhibition involves the dissociation of the P1 and chaperone domains of SurA and that the S220A and  $\Delta$ 215-216 mutations somehow destabilize the P1-core complex. In support of this, we find that SurA<sup>S220A</sup> is particularly protease susceptible, a possible indication that the disruption of interdomain contacts destabilizes SurA. This is corroborated by the reported instability of a SurA variant lacking both parvulin domains (14) and suggests that the P1 domain stabilizes and inhibits the core chaperone domain; a similar regulatory function has been proposed for the autoinhibited signaling adaptor protein Crk (36). Structural analysis has shown



**FIG 5** *bamB*-null phenotypes are suppressed by *surA10* and *surA13*. Whole-cell extracts of *bamA*<sup>+</sup> and *bamB::kan* strains (containing wild-type or suppressor alleles of *surA*) were analyzed by Western blotting using antisera that recognize LamB and OmpA. Detergent sensitivity phenotypes are shown below each lane; the qualitative growth score on agar plates containing SDS-EDTA is indicated by the number of “+” symbols present. –, no growth; ++, intermediate growth; +++, wild-type growth.



**FIG 6** SurA<sup>S220A</sup> is hyperactive *in vivo* and unstable *in vitro*. (A) Western blot analysis was performed with wild-type and *surA10* strains to determine relative levels of SurA, LamB, and OmpA. (B) Serial 10-fold dilutions of stationary-phase cultures of  $\Delta$ *surA* strains containing plasmids expressing SurA<sup>+</sup>, SurA<sup>S220A</sup>, SurA <sup>$\Delta$ P1</sup>, or SurA <sup>$\Delta$ P2</sup> were spotted onto LB with or without bacitracin and incubated at 37°C. (C) Periplasmic contents in cultures of wild-type and *surA10* strains were released by chloroform shock, and samples were incubated at 37°C prior to SDS-PAGE. SurA and the periplasmic protein MalE were detected by Western blotting using the appropriate antisera.

that the P1 domain can dissociate completely from the chaperone core while bound to a model peptide (14), suggesting that SurA may normally undergo conformational changes that involve the transient uncoupling of the P1 and core domains. The  $\alpha$ 2 helix of SurA, which contains the residues that are mutated in both *surA10* and *surA13*, forms multiple contacts with residues in the chaperone domain of SurA (13). It is possible, then, that mutations in P1 destabilize the P1-core complex, thereby eliminating an interaction that occludes both the P1 active site and a large surface in the core chaperone domain. It is tempting to suggest that BamAB catalyzes the displacement of P1 from the core domain.

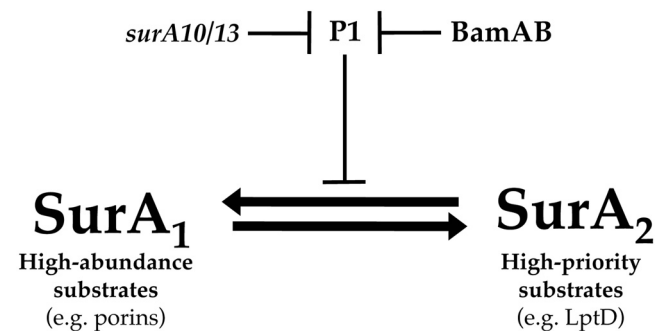
We favor a model in which SurA, by default, primarily assists the folding of high-priority substrates (such as LptD, which is principally dependent on SurA for assembly) (6) but can be induced by POTRA 1/2 and BamB to promote the assembly of abundant OMPs through modulation of its chaperone activity via the P1 domain. We suggest that the role of SurA in LptD biogenesis can be separated from its role as a general OMP chaperone and that BamA and BamB regulate these activities by adjusting the equilibrium between the autoinhibited and activated SurA conformations (Fig. 7).

An obvious advantage of this mode of regulation is that it permits selectivity by maintaining dedicated and semi-independent

OMP delivery pathways. This model provides a mechanism for both efficient assembly of highly abundant OMPs—several of which are present in numbers approaching 10<sup>5</sup> molecules/cell (37)—and the prioritized delivery of essential minor OMPs, such as LptD. This enables both the concentration-driven assembly of major OMP proteins and the selective assembly of low-abundance OMP species that are critical for cell growth.

Using this model as a framework, we suggest that the SurA variants described in this report effectively bypass a regulatory requirement for POTRA 1/2 and BamB by artificially relieving the P1-mediated autoinhibition of SurA chaperone activity (Fig. 7). Although this model provides a satisfying explanation for the genetic and biochemical data presented here, it does not explain why the P1 domain is required for suppression of *bamA616* (see Table S1a in the supplemental material). One possibility is that P1 performs a second role in addition to SurA autoinhibition; such a role is not without precedent, as cooperativity between proline isomerase and chaperone domains has been observed in several other bacterial PPIase-chaperone hybrid proteins, such as SlyD (30), trigger factor (31), and PrsA (38). If cooperative catalysis of protein folding occurs between P1 and the disinhibited SurA chaperone domain, this may account for the additional requirement for P1 in suppression of *bamA616* by *surA10* and *surA13*.

In addition to unmasking a regulatory function for a SurA PPIase domain, the model presented here has implications for the interplay between the factors comprising the *E. coli* periplasmic chaperone network. Recent work in our lab revealed an unexpected role for the secondary OMP chaperone Skp in assembly of LptD (39). According to the model diagrammed in Fig. 7, SurA conformational equilibrium should be shifted toward the form that favors assembly of LptD (SurA<sub>2</sub>) in a *bamB* mutant background. If this is the case, the profound synthetic LptD biogenesis defect that is observed upon simultaneous deletion of Skp and BamB (39) may in fact indicate a critical role for Skp in the prioritized assembly of LptD by SurA<sub>2</sub> in particular, or it may even suggest that Skp is somehow involved in shifting the equilibrium toward the SurA<sub>2</sub> conformation.



**FIG 7** Model for regulation of SurA activity by BamAB and the P1 domain of SurA. SurA exists in equilibrium between two conformations, one (SurA<sub>1</sub>) that is optimized for the targeting of major, abundant OMPs (such as OmpF, OmpC, and LamB), and another (SurA<sub>2</sub>) that promotes the prioritized assembly of critical, lower-abundance substrates (such as LptD). A shift in conformational equilibrium toward the SurA<sub>1</sub> state involves relief of P1-mediated inhibition of SurA chaperone activity through the combined action of BamB and the BamA N terminus. The *surA10* and *surA13* mutations bypass the requirement for BamAB in regulation of SurA activity, potentially by destabilizing the interaction between P1 and the core chaperone domain.

## MATERIALS & METHODS

**Bacterial strains and plasmids.** Strains and plasmids used in this study are described in Table S2 in the supplemental material. All strains were constructed using standard microbiological techniques. When necessary, media were supplemented with 125  $\mu$ g/ml ampicillin, 25  $\mu$ g/ml kanamycin, or 25  $\mu$ g/ml tetracycline. All bacterial cultures were grown under aerobic conditions at 37°C unless otherwise noted.

**Random and site-directed mutagenesis.** For a detailed description of mutagenesis procedures, see Materials and Methods in the supplemental material. Oligonucleotide primer sequences are provided in Table S3 in the supplemental material.

**Quantification of small molecule and antibiotic sensitivity.** Sensitivities to bacitracin, erythromycin, novobiocin, and rifampin were measured using the BBL Sensi-Disc antimicrobial susceptibility test discs (BD) as described (9). Susceptibility was quantified by measuring the diameter of the zones of inhibition around the discs. For efficiency of plating (EOP) assays, serial dilutions of stationary-phase cultures were spotted onto LB agar with or without 625  $\mu\text{g/ml}$  bacitracin and incubated overnight at 37°C.

**Genetic selection.** Spontaneous detergent-resistant suppressors of *bamA616* were obtained by plating 1 ml of an overnight culture at 37°C on LB agar containing SDS-EDTA (0.5%:1.0 mM). Suppressors that arose following overnight incubation were purified on selective media at 37°C. Mutations were mapped by P1 transduction and identified by DNA sequencing.

**Western blot analysis.** For a detailed description of Western blot procedures, see Materials and Methods in the supplemental material.

**Quantification of  $\beta$ -galactosidase activity.** Cells were grown in LB liquid medium with aeration at 37°C to mid-exponential phase. Aliquots of 1 ml were withdrawn from each culture, and cells were then pelleted and permeabilized by treatment with chloroform and SDS.  $\beta$ -Galactosidase activity from the chromosomal  $\sigma^E$ -dependent *rpoH<sub>P3</sub>::lacZ* reporter (26) was spectrophotometrically assayed in triplicate using the Miller assay (40) as previously described.

**Affinity purification and *in vivo* cross-linking.** For a detailed description of affinity purification and *in vivo* cross-linking procedures, see Materials and Methods in the supplemental material.

**Release of periplasmic proteins.** Periplasmic contents were released by chloroform shock as previously described (41). As an alternative method, cells were grown to an optical density at 600 nm ( $\text{OD}_{600}$ ) of 1, harvested by centrifugation, washed in 20 mM potassium phosphate (pH 7.2) and 150 mM NaCl, resuspended in BugBuster solution (Novagen) containing lysozyme (5  $\mu\text{g/ml}$ ), DNase I (50  $\mu\text{g/ml}$ ), RNase I (50  $\mu\text{g/ml}$ ), and allowed to lyse gently by rocking for 15 min at room temperature. Following lysis, the lysates were centrifuged at 10,000  $\times g$  for 10 min to remove debris. SDS-PAGE buffer was added to the samples, which were then boiled for 10 min prior to Western blot analysis as described above.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00540-13/-/DCSupplemental>.

- Text S1, PDF file, 0.1 MB.
- Text S2, PDF file, 0.1 MB.
- Figure S1, PDF file, 1 MB.
- Figure S2, PDF file, 0.3 MB.
- Figure S3, PDF file, 0.2 MB.
- Figure S4, PDF file, 2.2 MB.
- Figure S5, PDF file, 0.1 MB.
- Table S1, PDF file, 0.1 MB.
- Table S2, PDF file, 0.1 MB.
- Table S3, PDF file, 0.1 MB.

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