Supplemental Text

Identification of partial-loss-of-function alleles of *bamA*. Early characterization of BamA demonstrated the essentiality of the N-terminal periplasmic domain for Bam function and its role in mediating protein-protein interactions with the accessory lipoproteins BamB-E (28-30). Because of the obvious importance of this domain and the dearth of viable mutants available to probe its function, we sought to identify mutations in the periplasmic domain that compromise the activity of the machine. We restricted our mutational analysis of BamA to the periplasmic domain (POTRA 1-5) by conducting PCR-based mutagenesis of the portion of the *bamA* ORF that encodes the POTRA domains (residues 1-420). Mutagenized PCR product was digested and subcloned into pZS21::*bamA*, and the ligated products were introduced into JCM320, a strain in which expression of an ectopic, chromosomal wild-type allele of *bamA* can be induced by addition of arabinose. Only the plasmid-borne *bamA** allele is expressed in the JCM320/pZS21::*bamA** background when arabinose is excluded from the growth medium.

Mutations that compromise Bam function generally cause increased sensitivity to a variety of antibiotics and small molecules (23). Because defects in biogenesis of the OM are known to cause hypersensitivity to anionic detergents such as sodium dodecyl sulfate (SDS), we attempted to identify viable partial-loss-of-function alleles of *bamA* by screening pZS21::*bamA** transformants of JCM320 for SDS sensitivity. Using this approach, we isolated *bamA616* and a multitude of *bamA* mutations spanning the entire periplasmic domain.

***bamA616* increases OM permeability.** Many *bam* mutations cause increased sensitivity to multiple drugs and small molecules in addition to SDS (7, 9, 18, 22). To quantify the degree to which the OM permeability barrier is impaired in a *bamA616* mutant background, quantitative antibiotic susceptibility assays were performed using a panel of antibiotics with various properties (Table S1a). The *bamA616* mutant exhibits increased susceptibility to compounds that are normally excluded by the OM, particularly the antibiotic erythromycin.

This sensitivity indicates a weakened permeability barrier, a finding consistent with an unspecified defect in OM biogenesis.

The Cpx stress response is activated in the presence of *surA13*. Our observations suggest that the altered OMP profile in the *bamA616 surA13* suppressor strain is unlikely to reflect σ^E activity alone (see Fig. 3). We reasoned, therefore, that activation of a second extracytoplasmic stress response might underlie the unique properties of the *bamA616 surA13* double mutant.

In support of this, we observe a decrease in the abundance of the periplasmic sugar binding protein MalE in a *bamA616 surA13* background (Fig. S3a); this is surprising because MalE is not dependent on Bam or periplasmic OMP chaperones for its secretion or folding (2, 28; J.C. Malinverni, J. Werner, S. Kim, J.G. Sklar, D. Kahne, R. Misra, and T.J. Silhavy, Mol. Microbiol. **61**:151–64, 2006), nor is the *malE* gene it known to be a direct regulatory target of σ^E (25). This downregulation indicates modulation of a distinct signaling pathway controlling *malE* synthesis. We surmised that the CpxRA two component system might be responsible for this effect, as *malE*, *ompF*, and *lamB* expression are repressed upon induction of the Cpx stress response either directly via the response regulator CpxR or indirectly via Cpx-dependent cross-activation of the EnvZ/OmpR two-component system via the MzrA connector (H. Gerken, E.S. Charlson, E.M. Cicirelli, L.J. Kenney, and R. Misra, Mol. Microbiol. **72**:1408–22, 2009).

To test this, we first determined whether Cpx induction is sufficient to cause the change in OMP levels that we observed in the double mutant. We reasoned that if Cpx activation underlies the phenotypic differences between the *surA10* and *surA13* suppressors, then the *bamA616 surA13* phenotype should be reconstituted by artificially activating Cpx in the *bamA616 surA10* double mutant. To do this, we took advantage of a previously described mutation in *cpxA* that causes constitutive activation of Cpx (T.L. Raivio and T.J. Silhavy, J. Bacteriol. **179**:7724–33, 1997). This mutation, *cpxA24*, was introduced into the *bamA616 surA10* mutant and steady-state OMP levels were analyzed. We found that the activating *cpxA24* mutation is sufficient to decrease levels of LamB in a *bamA616 surA10* background such that they are equivalent with those observed in

bamA616 surA13 (Fig. S3b), showing that Cpx activation is sufficient to cause this decrease.

To prove that Cpx activation is necessary for the allele-specific *bamA616 surA13* phenotypes, we blocked Cpx signaling by removing the response regulator CpxR, the effector of the Cpx system. We reasoned that if Cpx signaling were responsible for the OMP and *malE* downregulation observed in *bamA616 surA13*, then abrogating Cpx signaling by deleting *cpxR* should restore expression of the downregulated factors. Indeed, we find that the introduction of a *cpxR* null mutation into the *bamA616 surA13* strain results in the restoration of LamB levels (Fig. S3c). Furthermore, *bamA616 surA10* and *bamA616 surA13* are both still SDS/EDTA^{R} when CpxR is deleted (data not shown). These data show that Cpx induction is not required for suppression of *bamA616* by *surA13*, and that the difference between the two *surA* suppressors reflects variability in Cpx activity. The Cpx-dependent downregulation of various factors in a *bamA616 surA13* background shows that the *surA13* mutation somehow causes Cpx induction. Because of the pleiotropic effects caused by the *surA13* allele, we hereafter concentrate our analysis on the *surA10* suppressor.

The *bamA616* mutation does not affect the BamAB interaction. BamB is the only Bam lipoprotein that requires multiple BamA POTRA domains to maintain a stable physical interaction with the Bam complex (30). We sought to determine whether the *bamA616* mutations disrupt the physical interaction between BamA and BamB, thereby creating an apparent BamB defect. To probe this directly, we affinity-purified His-BamA^{WT} and His-BamA^{616} and assessed the relative amount of co-purified BamB. We observe no difference in the amount of BamB that co-purifies with the mutant BamA^{616} protein relative to BamA^{WT} (Fig. 2, bottom panel). Similarly, the amount of co-purified BamC, which interacts indirectly with BamA through BamD (J.C. Malinverni, J. Werner, S. Kim, J.G. Sklar, D. Kahne, R. Misra, and T.J. Silhavy, Mol. Microbiol. **61**:151–64, 2006), is also equivalent between BamA^{WT} and BamA^{616}. We conclude that *bamA616* does not affect the physical association of BamA with the Bam lipoproteins. If the *bamB*-null-like phenotype of *bamA616* indeed reflects a loss of BamB function, it must not result from a defective interaction between BamA and BamB.

***surA10* is a gain-of-function mutation.** To determine whether *surA10* is a gain-of-function allele, we performed diploid analysis to assess the behavior of a *bamA616* strain containing wild-type SurA and $SurA^{S220A}$. We constructed *surA* diploid strains by introducing a pACYC177-derived vector expressing an arabinose-inducible wild-type allele of *surA* (8) into *bamA616 surA10*. The expression of $surA^+$ in this strain did not negate the suppressive effect of *surA10*; a *bamA616 surA10* strain remains SDS-resistant upon induction of wild-type *surA* expression (Table S1b). *surA10* can therefore be regarded as dominant to $surA^+$ in diploid analysis.

To better assess the relative activity of $SurA^{S220A}$, we replaced the wild-type chromosomal allele of *surA* in an otherwise wild-type (i.e. $bamA^+$) strain with *surA10* and characterized the resulting mutant by quantifying antibiotic resistance, determining levels of OMPs, measuring stress response activation, and determining the level of $SurA^{S220A}$ itself. We found that a *surA10* $bamA^+$ strain is as resistant to SDS and a variety of antibiotics as the wild type, levels of the model OMPs LamB and OmpA are unchanged, and levels of $SurA^{S220A}$ at steady state are comparable to $SurA^+$ (Table S1b, Fig. 6a).

Because *surA10* effectively substitutes for $surA^+$, we attempted to determine whether the S220A mutation causes subtle changes in the activity of SurA. We reasoned that slight differences in SurA activity might be unmasked by limiting the expression of *surA* beyond the threshold concentration for wild-type *in vivo* activity. To do this, we introduced the S220A mutation by site-directed mutagenesis into pSurA and measured the ability of $pSurA^+$ and $pSurA^{S220A}$ to complement a Δ*surA* chromosomal deletion. When expression of each construct is induced by addition of arabinose, both $SurA^+$ and $SurA^{S220A}$ fully complement the Δ*surA* deletion with respect to antibiotic sensitivity. When expression of *surA* is limited by excluding arabinose, basal transcription of the plasmid-borne allele sustains SurA concentrations that are 10- to 15-fold below that of chromosomally-expressed SurA (data not shown). Under these conditions, we find that $SurA^{S220A}$, but not $SurA^+$, confers wild-type resistance to bacitracin (Fig. 6b). This shows that *surA10* better maintains the OM permeability barrier at limiting concentrations, suggesting that the mutant

protein exhibits increased or altered activity relative to wild-type SurA. The dominance of *surA10* implies that it constitutes a gain-of-function allele of *surA*.

Because $SurA^{S220A}$ seems to possess a change in activity compared with $SurA^{+}$ we sought to rule out the possibility that an increase in SurA abundance would be sufficient to suppress the *bamA616* mutation. We overexpressed SurA in a *bamA616* strain and found that it is not sufficient to restore detergent resistance even when induced in the presence of arabinose, (Table S1b). Consistent with results described above, $pSurA^{S220A}$ transformants of *bamA616* were $SDS/EDTA^{R}$ even in the absence of arabinose, confirming that *surA10* is a potent, dominant suppressor of *bamA616* (Table S1b). This suggests that the S220A mutation impacts the activity of SurA in a manner that cannot be replicated by simply increasing SurA levels.