

A Suppressor Mutation That Creates a Faster and More Robust σ E Envelope Stress Response

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ABSTRACT

The σ E envelope stress response is an essential signal transduction pathway which detects and removes mistargeted outer membrane (OM) β -barrel proteins (OMPs) in the periplasm of *Escherichia coli*. It relies on σ E, an alternative sigma factor encoded by the *rpoE* gene. Here we report a novel mutation, a nucleotide change of C to A in the third base of the second codon, which increases levels of σ E (*rpoE_S2R*). The *rpoE_S2R* mutation does not lead to the induction of the stress response during normal growth but instead changes the dynamics of induction upon periplasmic stress, resulting in a faster and more robust response. This allows cells to adapt faster to the periplasmic stress, avoiding lethal accumulation of unfolded OMPs in the periplasm caused by severe defects in the OMP assembly pathway.

IMPORTANCE

Survival of bacteria under conditions of external or internal stresses depends on timely induction of stress response signaling pathways to regulate expression of appropriate genes that function to maintain cellular homeostasis. Previous studies have shown that strong preinduction of envelope stress responses can allow bacteria to survive a number of lethal genetic perturbations. In our paper, we describe a unique mutation that enhances kinetics of the σ E envelope stress response pathway rather than preinducing the response. This allows bacteria to quickly adapt to sudden and severe periplasmic stress.

Maintaining the integrity of the cell envelope is an essential function in bacteria. In the case of Gram-negative bacteria, such as *Escherichia coli*, the inner membrane (IM) and outer membrane (OM) delimit the periplasm, an extracytoplasmic compartment that is not accessible to cytoplasmic protein quality control mechanisms (1). Accordingly, detection and removal of mistargeted OM β -barrel proteins (OMPs) in the periplasm rely on a dedicated signal transduction system known as the σ E envelope stress response (2). At the center of this response is σ E (σ 24 or RpoE), an alternative sigma factor encoded by the *rpoE* gene, which is cotranscribed with *rseABC* from a σ 70 (RpoD)-dependent promoter (Fig. 1) (3). The *rseA* gene encodes an anti-sigma factor, which binds to and inhibits RpoE activity during normal growth (4, 5).

Induction of σ E activity in response to stress is regulated at the level of RseA degradation and has become a paradigm for regulated intramembrane proteolysis (Fig. 1) (2). RseA is an IM protein with a cytoplasmic domain that is responsible for binding and inhibiting σ E (4, 5). When the OMP assembly pathway is compromised, misfolded OMPs accumulate in the periplasm and expose their C-terminal YXF motif to the IM protease DegS (6). Binding of these motifs to a periplasmic PDZ domain of DegS activates its proteolytic activity and results in the first cut of RseA (6–8). This event induces a further proteolytic cascade, including a cut within the RseA transmembrane domain by the IM protease RseP (8, 9) and degradation of the remaining RseA cytoplasmic domain by cytoplasmic proteases, such as Clp (10). When σ E is released, it directs RNA polymerase to transcribe a specific subset of genes: the well-characterized σ E regulon (11). Expression of small RNAs (sRNA), such as MicA and RybB, is induced, resulting in translational downregulation of OMP synthesis (12, 13). This reduces the load on the OMP assembly pathway and minimizes further accumulation of unfolded OMPs in the periplasm.

Moreover, σ E also upregulates the expression of the OM biogenesis machines and periplasmic chaperones to promote OMP folding as well as periplasmic proteases to degrade misfolded OMPs (11).

In addition, σ E increases transcription of its own operon from a σ E-dependent promoter, as well as transcription of *rseABC* from an additional σ E-dependent promoter located within *rpoE* (Fig. 1) (11). This creates a positive-feedback loop that persists as long as the stress continues and RseA is cleaved. When periplasmic stress is removed, RseA degradation is inhibited, and the accumulated anti-sigma factor binds and inhibits the activity of σ E. Although *rpoE* and *rseA* lie in the same operon, recent studies performed using ribosome profiling have shown that RseA is made in excess of σ E (14), ensuring that σ E activity is tightly inhibited in the absence of periplasmic stress.

In this paper, we report a novel suppressor mutation in *rpoE* that allows cells to survive lethal periplasmic stress. The suppressor mutation increases production of σ E and RseA, thereby priming cells for a faster and more potent envelope stress response.

Received 21 April 2016 Accepted 14 June 2016

Accepted manuscript posted online 20 June 2016

Citation Konovalova A, Schwalm JA, Silhavy TJ. 2016. A suppressor mutation that creates a faster and more robust σ E envelope stress response. *J Bacteriol* 198:2345–2351. doi:10.1128/JB.00340-16.

Editor: R. L. Gourse, University of Wisconsin—Madison

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Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JB.00340-16>.

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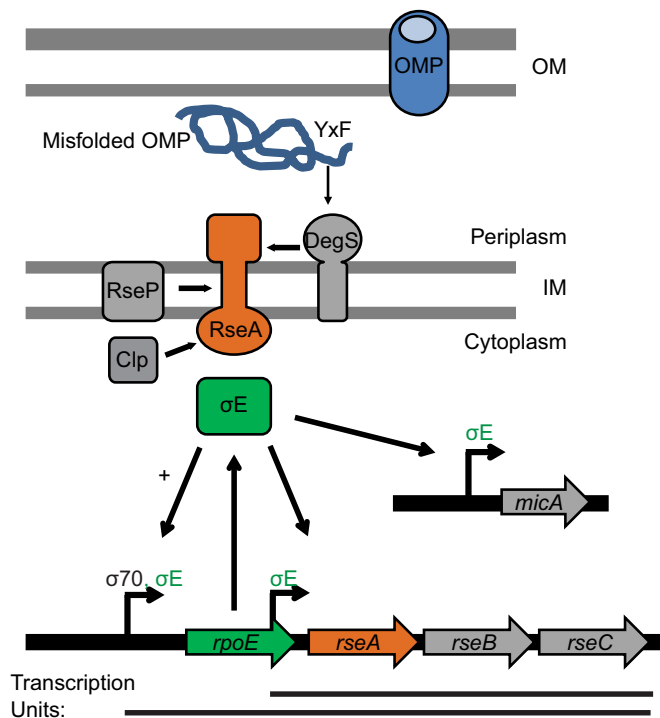


FIG 1 σE stress response pathway. σE is encoded by the *rpoE* gene, which is transcribed with *rseABC* from a $\sigma 70$ promoter. RseA is an IM protein, which binds and inhibits σE . When the OMP assembly pathway is compromised, OMPs misfold in the periplasm and a YXF motif activates the DegS protease. DegS initiates the proteolytic pathway, and RseA is further degraded by the RseP and Clp proteases. σE is released and activates expression from σE -dependent promoters, e.g., those of the *micA* gene and the *rpoE-rse* and *rseABC* operons.

MATERIALS AND METHODS

All strains used in this study are listed in Table S1 in the supplemental material.

Suppressor analysis of the OMP assembly double mutants. A spontaneous suppressor of the synthetic lethal phenotype of the *surA degP* double mutant was isolated through selection for growth at 37°C (*rpoE_S2R*). The mutation was mapped by identifying linked Tn10 insertions and sequence analysis of suspected target genes. *rpoE_S2R* linked to *nadB::Tn10* was then transduced into P_{BAD} -*surA* or P_{BAD} -*bamE* depletion strains, carrying additional *degP*, *skp*, or *bamB* mutations. Viability of these strains was tested by an efficiency-of-plating assay. For this assay, strains were grown overnight in LB supplemented with 0.2% arabinose and washed in LB to remove arabinose, and 10-fold serial dilutions were plated on the LB agar with or without arabinose and incubated at 37°C overnight.

β -Galactosidase assay. Strains were grown at 37°C unless otherwise indicated. A 100- μ l volume of mid-log culture (optical density at 600 nm [OD_{600}], 0.5 to 0.7) was taken and added directly to 900 μ l of Z buffer (60 mM Na_2HPO_4 , 40 mM NaH_2PO_4 , 10 mM KCl, 1 mM $MgSO_4$, 50 mM β -mercaptoethanol, 0.03% SDS). A 50- μ l volume of chloroform was added to stop growth, and the reaction mixture was mixed vigorously by pipetting. A 100- μ l volume of each cell lysate was mixed with 100 μ l of 4 mg/ml ONPG (O-nitrophenyl- β -D-galactopyranoside) solution in Z buffer. β -Galactosidase activity was analyzed by a kinetic measurement of the OD_{420} in a BioTek Synergy 1 plate reader, and V_{max} was determined using Gen5 software. The V_{max} value was normalized by OD_{600} . Experiments were performed in three biological replicates, and mean values \pm standard errors of the means (SEM) of the results were plotted. Graphs were built using GraphPad Prism 6 software.

Immunoblot analysis. Strains were grown at 37°C until mid-log phase (OD_{600} , 0.5 to 0.7). Samples were normalized by determination of OD_{600} . Anti-RpoE antibodies (Neoclone) were used for immunoblotting. RpoE levels were normalized to the loading control and quantified relative to the wild type (WT) using ImageJ software.

Determination of the rate of translation initiation of the reporter fusions. Overnight cultures of corresponding strains containing reporter plasmids or empty vector controls were diluted 1:500 in LB supplemented with 0.2% arabinose and ampicillin. Strains were grown in a 200- μ l volume in 96-well black plates with clear bottoms (Costar) at 37°C and OD_{600} and green fluorescence (excitation, 481 nm; emission, 507 nm) and red fluorescence (excitation, 580 nm; emission, 610 nm) were monitored every 20 min using a BioTek Synergy 1 plate reader. Each strain was grown in six wells to avoid plate effects, and mean values for these replicates were calculated. The background fluorescence of the empty vector control was subtracted from the fluorescence of strains containing reporter fusions. This specific fluorescence was normalized for OD_{600} and plotted as a function of time. Experiments were performed three independent times, and a plot of the mean values \pm SEM is shown in Fig. S1 in the supplemental material.

Linear regression analysis was performed on the graphs within the linear range of 300 to 480 min, with R^2 values of 0.98 ± 0.07 (standard deviation [SD]) for green fluorescent protein (GFP) fluorescence graphs and 0.97 ± 0.02 (SD) for mCherry fluorescence graphs. *gfp* or *mCherry* translation rates were taken as the slope of the line and calculated for each of the biological replicates separately. The ratio between the *mCherry* and *gfp* translation rates was determined and calculated as the fold change from the rate determined for the WT-*gfp* of WT-*mCherry* construct. These values are plotted as a bar graph with mean values \pm SEM. Significance analysis was performed using an unpaired *t* test, and differences were considered to be significant with *P* values of ≤ 0.05 . Exact *P* values for significantly different pairs are shown on the graph (see Fig. 3C).

Determination of promoter activity. GFP fluorescence normalized to the OD_{600} during steady-state growth was used to test differences in expression of the *PrpoE-gfp* and *PmicA-gfp* reporter fusions in *rpoE_S2R* strains versus WT strains. Strains were grown to mid-log phase (OD_{600} of 0.5), and GFP fluorescence (excitation, 481 nm; emission, 507 nm) was measured in a 200- μ l volume using a BioTek Synergy 1 plate reader and blank normalized against the strain lacking GFP reporters. This experiment was performed three independent times, and a plot of mean values \pm SEM is shown (see Fig. 3D). Significance analysis was performed pairwise using an unpaired *t* test, and differences were considered to be significant with *P* values of ≤ 0.05 . Exact *P* values for significantly different pairs are shown on the graph (see Fig. 3D).

To determine the kinetics of σE induction in response to OmpC_YYF peptide expression, the corresponding strains were grown overnight in LB supplemented with corresponding antibiotics and 0.2% fucose to avoid the expression of the OmpC_YYF peptide. Cells were washed in LB and diluted 1:500 in LB supplemented with corresponding antibiotics, 0.5% glucose, and 0.2% arabinose for autoinduction. Strains were grown in a 200- μ l volume in 96-well black plates with clear bottoms (Costar) at 37°C and OD_{600} , and green fluorescence was monitored every 20 min using a BioTek Synergy 1 plate reader. Each strain was grown in 12 wells to avoid plate effects, and mean values for these replicates were calculated. The background fluorescence of the “no-*gfp*” control was subtracted from the fluorescence of strains containing reporter fusions. The increase of the specific fluorescence was plotted as a function of growth (OD_{600}), resulting in a differential plot within the window of exponential growth (see Fig. S2 in the supplemental material). The experiment was performed three independent times; a plot of mean values \pm SEM is shown (see Fig. 4), and corresponding growth curves (mean $OD_{600} \pm$ SEM over time) are shown (see Fig. S2). Note that the OD_{600} values are those obtained from micro-well reads and have not been path length corrected for a 1-cm-path-length cuvette.

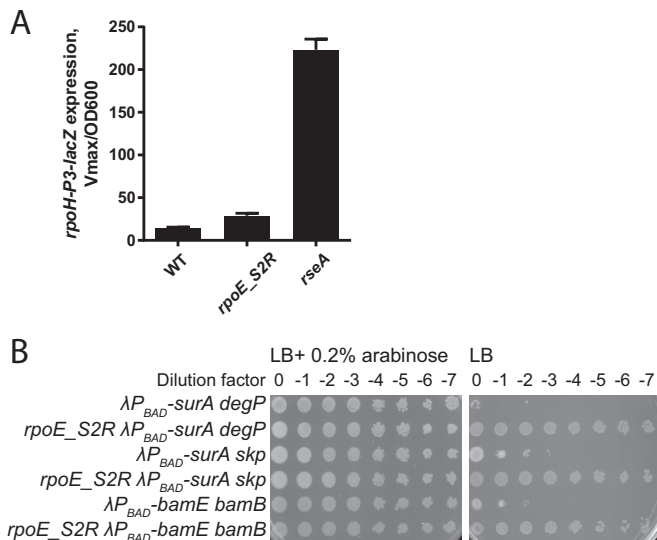


FIG 2 *rpoE_S2R* is a suppressor of synthetic double mutants in OMP assembly pathway. (A) Null mutations in *rseA* but not *rpoE_S2R* increase basal activity of σ E based on expression of the *rpoH-P3-lacZ* reporter. Graphs represent mean β -galactosidase activity \pm SEM; $n = 3$. (B) Chromosomal arabinose-dependent depletion constructs of *surA* or *bamE* were combined with null mutations in the *degP*, *skp*, or *bamB* genes. Strain viability was assayed by plating 10-fold serial dilutions of overnight cultures grown in LB with 0.2% arabinose and washed to remove arabinose before plating. *rpoE_S2R* fully rescued growth of the strains in the absence of arabinose.

RESULTS

***rpoE_S2R* is a powerful suppressor of a number of synthetically lethal combinations.** Simultaneous loss of the major periplasmic chaperone SurA and the periplasmic chaperone/protease DegP confers a strong synthetic phenotype caused by a severe OMP assembly defect, evidenced by the accumulation of unfolded OMPs in the periplasm (15, 16). The *surA degP* double mutant is viable only at 24°C. A search for suppressors that would allow growth of the *surA degP* double mutant at 37°C yielded null mutations in *rseA*. This class of suppressors allows survival because the σ E response is highly induced in this strain (Fig. 2A), resulting in the downregulation of OMP synthesis and upregulation of alternative chaperones and the OM biogenesis machines. This minimizes the periplasmic stress caused by the shift to the nonpermissive temperature.

We also found a mutation in the open reading frame (ORF) of *rpoE*, which changes the second codon from Ser (AGC) to Arg (AGA). Unlike in *rseA* null strains, steady-state levels of the σ E response were not increased (Fig. 2A). Despite this, *rpoE_S2R* is a powerful suppressor and fully restores normal growth at 37°C of the *surA degP* strain, as well as of other strains with synthetically lethal mutations, such as *surA skp* and *bamB bamE* (Fig. 2B).

Intrigued by the finding that the σ E response was not preinduced in the *rpoE_S2R* strain, we chose this mutant for further study in order to understand the mechanism of suppression.

***rpoE_S2R* upregulates expression of *rpoE* without induction of the σ E response.** Levels of σ E are quite low during normal growth in the WT background; however, levels were increased 8-fold in the *rpoE_S2R* mutant (Fig. 3A). *rpoE_S2R* has a nucleotide change of C to A in the third base of the second codon. The nucleotide sequence of the second codon has been shown to be

important for translation initiation (17–19). In *E. coli*, all codons have been previously tested for their effect on translation initiation based on reporter assays (19). Interestingly, the Arg codon AGA was found to be one of the best-performing codons, conferring increased translation rates compared to the Ser AGC codon (19).

To test whether the *rpoE_S2R* mutation resulted in translational upregulation of *rpoE*, we generated reporter plasmids that encode *rpoE*-translational fusions to *gfp* and *mCherry* genes that are under the control of arabinose-inducible P_{BAD} promoter. Translation of both *gfp* and *mCherry* fusions is initiated by the native ribosome-binding site (RBS) of *rpoE*, and each of the genes is translationally fused to the five initial codons of WT *rpoE*, resulting in WT-*gfp* and WT-*mCherry* fusions. We generated the second-codon mutations in either *rpoE-gfp* or *rpoE-mCherry* and then plotted the increase of red or green fluorescence normalized to the OD₆₀₀ as a function of time (see Fig. S1 in the supplemental material). *gfp* or *mCherry* translation rates were determined by calculating the slope of the line in these plots. The ratio between the corresponding *mCherry* and *gfp* translation rates was set to 1 in the reporter strain containing WT-*gfp* and WT-*mCherry* fusions. The effects of the various second-codon mutations are expressed as the fold change of this ratio and are plotted as a bar graph (Fig. 3C).

Figure 3C shows that the translation rates of S2R(AGA) were 1.5-fold to 2-fold higher than those of the WT, regardless of whether the S2R(AGA) mutation was introduced into the *gfp* or *mCherry* reporter construct, and the results showed an increase of about 2-fold to 3-fold in final fluorescence (see Fig. S1A and B in the supplemental material). To test, whether it was the nucleotide change rather than the amino acid change that was responsible for this effect, we used an alternative Arg codon, AGG, which occurs at the same frequency in the *E. coli* genome as AGA. The S2R(AGG) mutation did not cause the same increase in the translation rate as S2R(AGA). We therefore concluded that it was the C6A nucleotide change and not the S2R amino acid change that was responsible for translational upregulation.

To determine if the increased *rpoE* translation rate caused by the *rpoE_S2R* mutation causes induction of the σ E response, we monitored the expression of two previously published transcriptional reporters, *PrpoE-gfp* and *PmicA-gfp* (Fig. 3D). The *PrpoE-gfp* fusion is the most sensitive reporter for a change in σ E protein levels (20). This fusion does not contain the native *rpoE* RBS or part of the *rpoE* ORF and therefore reports exclusively on the transcription. The *micA* gene is a member of the σ E regulon; it encodes a small RNA that functions to downregulate OMP synthesis. *PmicA-gfp* is the most sensitive promoter reporter for the σ E regulon (20).

During logarithmic growth, the expression of *PrpoE-gfp* was about 3-fold higher than that of the WT (Fig. 3D). This increased *PrpoE* promoter activity also contributed to increased σ E levels. However, increased transcription from this promoter also resulted in increased levels of RseA, which would keep σ E inhibited. Consistent with this increase in RseA, the σ E response was not induced, as judged by lack of significant increase in the activity of the *PmicA* promoter (Fig. 3D), and was in line with what we observed using the *rpoH-P3-lacZ* reporter (Fig. 2A).

***rpoE_S2R* changes the kinetics of the response to periplasmic stress.** Our genetic analysis suggested that preinduction of the σ E response cannot explain the suppression caused by *rpoE_S2R*. For

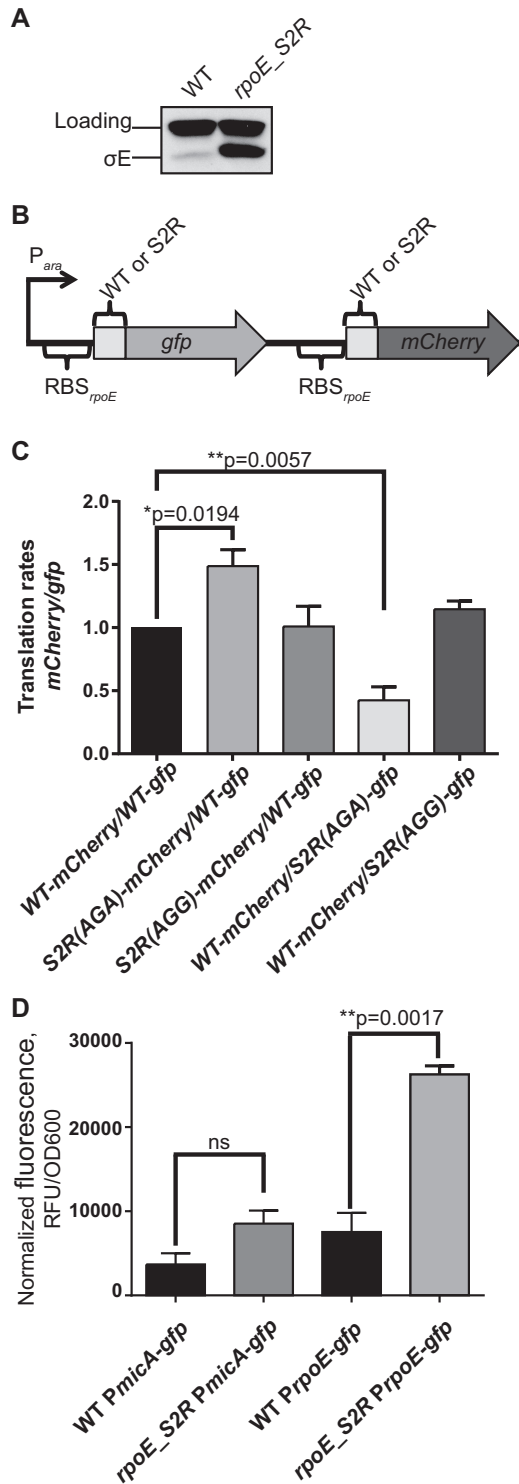


FIG 3 *rpoE_S2R* upregulates expression of *rpoE*. (A) Levels of the σE protein were significantly increased in an *rpoE_S2R* mutant during steady-state growth. (B) Organization of translational reporter construct. *gfp* and *mCherry* genes were translationally fused with the first five codons of *rpoE*. The expression of both genes was controlled by an arabinose-inducible promoter and the native *rpoE* RBS. *S2R* mutations (AGA or AGG codons) were introduced in either the *gfp* or *mCherry* gene fusions. (C) The *S2R*(AGA) mutation significantly increased the translation initiation rate. This graph represents the ratio of the translation rates of *mCherry* and *gfp*, normalized to the WT-*gfp*/WT-*mCherry* construct. Means \pm SEM are indicated with *P* values for the con-

example, the presence of *rpoE_S2R* does not lead to increased σE activity in the *degP* mutant (see Fig. S2A in the supplemental material); however, the *surA* mutation can be introduced only in the *degP rpoE_S2R* strain and not in the *degP* strain at 37°C. When grown at room temperature, the *degP surA* strain induces σE activity very strongly (even more strongly than the *degP surA rpoE_S2R* strain). This suggests that *rpoE_S2R* allows the *degP surA* strain to grow and that the growth is not due to higher levels of initial or endpoint σE activity but is perhaps due to the fact that the σE is induced more rapidly. We reasoned that the increased levels of σE might be responsible for suppression because they would change the kinetics of the response to periplasmic stress through an enhanced positive-feedback loop.

During steady-state growth, the mutants described above were adapted to chronic periplasmic stress, by readjusting the gene expression of OMPs as well as of the biogenesis machines. Therefore, we sought an experimental approach in which we could transiently generate periplasmic stress in an otherwise-WT background and then follow the kinetics of σE induction in the *rpoE_S2R* strain versus the WT. For this, we used an OmpC-derived C-terminal peptide, which acts as an inducing signal for DegS (6). For this assay, we generated a plasmid that directs periplasmic expression of the OmpC_YYF peptide under the control of the arabinose-inducible P_{BAD} promoter according to the method described in reference 6. This plasmid was introduced into the WT or *rpoE_S2R* strain containing either the *PrpoE-gfp* or the *PmichA-gfp* reporter. We then monitored induction of the σE response by following the increased production of GFP as a function of growth. To induce expression of the OmpC_YYF peptide, we used an autoinduction approach that was based on catabolite repression. We grew strains in LB containing glucose and arabinose. In the presence of glucose, expression of P_{BAD} is tightly repressed (21). This avoids any leaky production of the OmpC_YYF peptide and therefore avoids any preinduction of the σE response. Upon glucose depletion through the cell growth, arabinose should rapidly induce expression of the P_{BAD} promoter, resulting in the production of the OmpC_YYF peptide. First, we tested this autoinduction approach in a WT background (Fig. 4, upper panels). In the presence of glucose, at a lower OD₆₀₀, reporter activity was present at similarly low levels, regardless of the presence or absence of the OmpC_YYF construct. Once cultures reached an OD₆₀₀ of 0.5 and glucose levels became depleted, the activity of both the *PrpoE* promoter and the *PmichA* promoter increased as expected in an OmpC_YYF-dependent manner, indicating that the σE response was induced under those conditions.

We next compared the kinetics of σE induction in an *rpoE_S2R* strain to the kinetics in the WT. Figure 4 (lower panels) shows that once cultures reached an OD₆₀₀ of 0.5, both reporters were induced more rapidly and also to much higher levels in the *rpoE_S2R* strain than in the WT background (note the scale difference between the upper and lower panels of Fig. 4). Therefore, we concluded that the *rpoE_S2R* strain responds more quickly and

structs with significant changes in ratios as determined using a *t* test. (D) *PrpoE* but not *PmichA* promoter activity was increased in the *rpoE_S2R* strain. This graph represents the normalized fluorescence of GFP expressed from *PrpoE* or *PmichA* promoters during mid-logarithmic growth of the WT or *rpoE_S2R* strains. Means \pm SEM are indicated with *P* values for the constructs with significant changes in GFP expression as determined using a *t* test. RFU, relative fluorescence units.

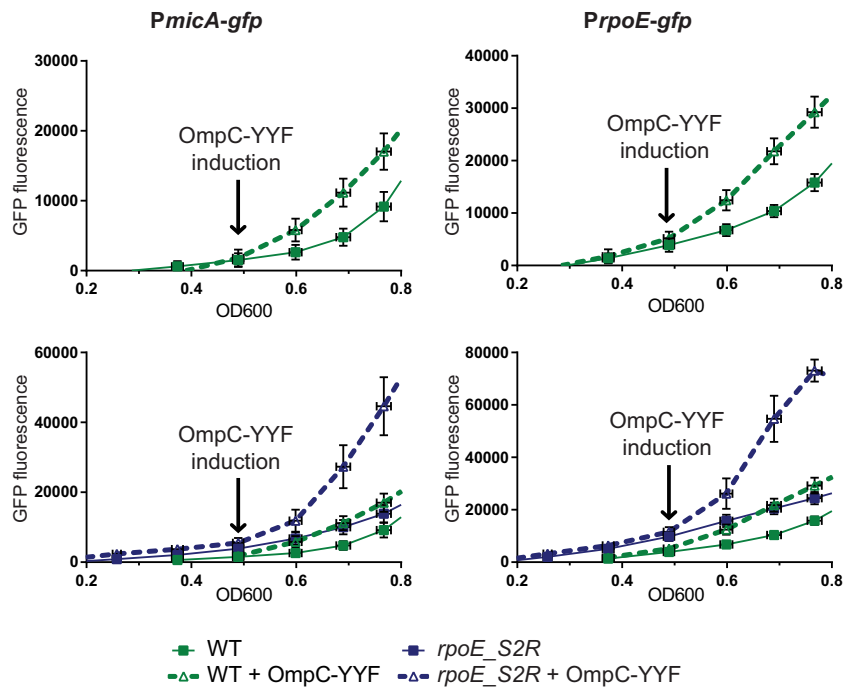


FIG 4 Kinetics of σ E induction monitored by GFP expression from *PrpoE*- or *PmicA*-*gfp* promoter fusions. Strains were grown in LB with glucose and arabinose in order to create autoinduction of OmpC-YYF peptide expression (where applicable). Graphs represent a differential plot of the increase of fluorescence as a function of growth (means \pm SEM). Corresponding growth curves are included as described for Fig. S2 in the supplemental material. Induction of OmpC-YYF peptide at an OD_{600} of 0.5 led to the induction of expression of the *PrpoE*- or *PmicA*-*gfp* transcriptional reporters. The upper graphs show induction in the WT background. The lower panels show induction in *rpoE*_{S2R} compared to the WT results (note scale difference).

more robustly to periplasmic stress, allowing cells to combat the resulting damage before it reaches lethal levels.

DISCUSSION

In this paper, we report a novel mutation, *rpoE*_{S2R}, that suppresses the synthetic lethality observed with double mutant strains lacking components of the OMP assembly pathway. The *rpoE*_{S2R} mutation increases the levels of σ E by increasing the translation rate. Strikingly, however, the *rpoE*_{S2R} mutation does not increase the activity of σ E but instead changes the dynamics of σ E induction upon periplasmic stress, resulting in a faster and more robust response. When a cell encounters stress, in order to survive, it must mount an effective defense that prevents damage from reaching lethal levels. The faster and more robust σ E response caused by the *rpoE*_{S2R} suppressor mutation allows cells to win the race against the periplasmic stress caused by the crippled OMP assembly pathways.

How does *rpoE*_{S2R} change the dynamics of the σ E stress response? In WT cells, σ E is maintained at very low levels during vegetative growth. Although *rpoE* and *rseA* are located in the same operon, RseA is made in excess of σ E (14), likely because *rpoE* possesses a weak RBS that is suboptimally positioned relative to the translation start site. Because a positive-feedback loop is incorporated into the σ E pathway, an excess of RseA makes the pathway noise resistant. We found that levels of σ E are significantly increased in the *rpoE*_{S2R} strain for two reasons. First, the S2R mutation directly increases RpoE levels by enhancing translation. Using a translational reporter, we showed that the nucleotide change C6A in *rpoE* results in an increased translation rate. We show that this increase is due to the nucleotide change and not

the amino acid change, because another Arg codon failed to support increased translation. Although we did not address the mechanisms for translation enhancement, it has been previously shown that the nucleotide sequence of the second codon affects translation initiation and that this is especially important in cases of leaderless mRNAs or genes with weak RBSs (17–19). Second, the *rpoE*_{S2R} mutation indirectly increases RpoE levels by increasing transcription of the *rpoE*-*rseA* operon from the σ E-dependent promoter. This increased transcription from the *PrpoE* promoter (Fig. 1) would also increase RseA levels, and this, in turn, would inhibit σ E, preventing the induction of the stress response. Although we have not determined the ratio of RseA to σ E in the *rpoE*_{S2R} strain, we think that it is likely that the levels of σ E relative to RseA are somewhat increased because of the increased transcription from *PrpoE*. However, this increase is apparently not sufficient to support an increase in the expression of regulon genes, as evidenced by the lack of a significant increase in the activity of the *PmicA* or *rpoH*-P3 promoters.

We demonstrated that the increased levels of σ E dramatically affect the kinetics of the stress response. Unlike in WT cells, RseA is made in excess of σ E. When cells experience periplasmic stress, similar degradation rates of RseA would release more σ E molecules in the *rpoE*_{S2R} strain than in the WT. Because the activity of σ E is directly linked to the number of free (uninhibited by RseA) molecules, the release of more molecules of σ E results in faster activation of the response. In addition, the increased translation rate of *rpoE*_{S2R} would result in faster signal amplification through the positive-feedback loop, which would result in a response much stronger than that seen in the WT.

Mutations that increase the basal activity of the envelope stress responses are able to suppress a number of lethal genetic permutations. For example, a null mutation in *rseA*, which leads to full activation of the σE pathway during steady-state growth, can also suppress the *surA degP*, *surA skp*, *bamE bamB* (our work), and *degP bamB* (22) synthetically lethal double mutants. This is because OMP expression is significantly reduced in this strain, bypassing the requirement for certain nonessential components in the OMP pathway that function to increase the efficiency of the assembly reaction. Consistent with this, mutations in the EnvZ/OmpR two-component signal transduction pathway, which also lead to decreased expression of abundant OMPs, also suppress *degP bamB* synthetic lethality (23). Similarly, mutations such as *cpxA**, which causes constitutively high activity of the Cpx envelope stress response, can suppress lethality caused by toxic Lamb-LacZ fusions or misfolded P pilus subunits (24, 25). In this respect, the *rpoE_S2R* mutation is unique, because the mechanism of suppression does not depend on the preinduction of the stress response but instead relies on enhanced kinetics, which allows the σE response to win the race against increasing periplasmic stress.

If the *rpoE_S2R* mutation creates a faster, more robust stress response, why then has evolution apparently selected against it? On the basis of the results of our experiments with the OmpC_YYF peptide, we suggest that the *rpoE_S2R* strain overreacts to small stresses. This may come with a fitness cost, because high-level induction of the σE pathway can be detrimental to cells (26–28). These harmful effects are caused at least in part by destabilization of the OM due to decreased OMP levels (29). In addition, decreased OMP levels result in increased sensitivities to detergents and antibiotics (30, 31). Because of these defects in OM integrity, it is likely that the *rpoE_S2R* mutation would be detrimental to the survival of *E. coli* in its natural environment.

ACKNOWLEDGMENT

We thank Carol A. Gross (UCSF) for providing *PrpoE*- and *PmicA-gfp* reporter plasmids.

FUNDING INFORMATION

This work, including the efforts of Thomas J. Silhavy, was funded by HHS | NIH | National Institute of General Medical Sciences (NIGMS) (GM34821).

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