RfaH Suppresses Small RNA MicA Inhibition of fimB Expression in Escherichia coli K-12

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The phase variation (reversible on-off switching) of the type 1 fimbrial adhesin of Escherichia coli involves a DNA inversion catalyzed by FimB (switching in either direction) or FimE (on-off switching). Here, we demonstrate that RfaH activates expression of a FimB-LacZ protein fusion while having a modest inhibitory effect on a comparable fimB-lacZ operon construct and on a FimE-LacZ protein fusion, indicating that RfaH selectively controls fimB expression at the posttranscriptional level. Further work demonstrates that loss of RfaH enables small RNA (sRNA) MicA inhibition of fimB expression even in the absence of exogenous inducing stress. This effect is explained by induction of α5, and hence MicA, in the absence of RfaH. Additional work confirms that the procaine-dependent induction of micA requires OmpR, as reported previously (A. Coornaert et al., Mol. Microbiol. 76:467–479, 2010, doi:10.1111/j.1365-2958.2010.07115.x), but also demonstrates that RfaH inhibition of fimB transcription is enhanced by procaine independently of OmpR. While the effect of procaine on fimB transcription is shown to be independent of RcsB, it was found to require SlyA, another known regulator of fimB transcription. These results demonstrate a complex role for RfaH as a regulator of fimB expression.

Like many adhesins, type 1 fimbriation is controlled by phase variation (the reversible on-off switching in gene expression that produces a mixed population). fim phase variation involves the site-specific inversion of an ~300-bp promoter element (fimS) that contains a promoter for the fimbrial structural genes (1). Inversion is catalyzed by recombinases FimE (on-off switching) and FimB (low-frequency switching in either direction), encoded by genes situated adjacent to fimS (2), as well as by alternative recombinases situated elsewhere in the chromosome in some pathogenic strains (3, 4).

RfaH to a paused RNA polymerase (RNAP) transcription elongation complex (13, 23). The ability of RfaH to switch between transcriptional regulator and translational activator involves an unprecedented refolding of the RfaH carboxy-terminal domain (RfaH-CTD) from an all-α to all-β confirmation, enabling RfaH to bind to ribosomal protein S10 (13). fimB, which has a sigma 70 promoter, has a large (271-bp) 5′ untranslated region (5′ UTR), suggesting that the recombinase gene may be subject to extensive control following transcription initiation (24, 25). While this possibility has yet to be investigated in detail, fimB expression has been shown recently to be inhibited by the α5-dependent small RNA (sRNA) MicA (26). Here, we report that fimB expression is also enhanced by RfaH and that this effect requires MicA.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this work are listed in Table 1. All bacterial strains are derivatives of E. coli K-12. To combine mutant fimB alleles with a FimB-LacZ protein fusion, PCR-generated mutant DNAs were first cloned into plasmid pIB347, a derivative of the temperature-sensitive vector pMAK705 (27), to replace the wild-type region of E. coli K-12. To replace the SphI-ClaI region. Allelic exchange was then used to transfer the mutations from the plasmids into the chromosomal fim region of strain BGE043 or KCEC840 using sacB and sucrose counterselection as

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described previously (28). A *micA-lacZYA* fusion replacing the wild-type *micA* gene was constructed by inserting an XbaI fragment containing the promoterless *lacZYA* genes isolated from plasmid pLB341 into a *micA* vector (pAM011) to generate pAM012. The *micA-lacZYA* construct was then transferred into the genome by allelic exchange between pAM012 and strain AAEC100 (MG1655 *lacZYA*) (28). Strain AAEC189 (*Δfim*) was used as the host strain for recombinant plasmids to ensure that the DNA was suitably methylated to allow subsequent transformation of the strain MG1655 (23). P1 transduction was carried out using P1_{vir} as described previously (29).

**Media and growth conditions.** The media included L broth (5 g of sodium chloride, 5 g of yeast extract, and 10 g of tryptone per liter [Difco]) and L agar (L broth with 1.5% agar [Difco]). Sucrose agar, used to select recombinant bacteria (13), is L agar supplemented with 6% sucrose in the absence of sodium chloride. The antibiotics chloramphenicol (25 μg/ml) and kanamycin (25 μg/ml) were included in selective media as required (Sigma). Lactose MacConkey plates (BD) were used as an indicator medium to determine the proportion of switch-on to switch-off bacteria. For rich defined (RD) medium, minimal MOPS [3-(N-morpholino)propanesulfonic acid] medium was prepared as described by Neidhardt et al. (30). The media were supplemented with 10 mM thiamine, 0.4% glycerol, bases, vitamin B supplement, and amino acids as reported originally by Neidhardt et al. (30). In experiments that included an *rseA* mutant control, the medium was supplemented with 1 mM nicotinic acid. All re-

**TABLE 1 Strains used in this study**

| Strain       | Relevant characteristics | Source/reference |
|--------------|--------------------------|------------------|
| MG1655       | K-12 wild type; λ F Imm    | E. coli Genetic Stock Center (CGSC) (47) |
| AAEC189      | YMC9 (λ F supE44 hsdR17 mcrA mcrB end A1 thi 1ΔargF-lac-205 ΔfimB-H ΔrseA) | 29 |
| JW0052-1     | BW23113 ΔracAHKan'        | CGSC/Keio collection (48) |
| JW2205       | BW23113 Δ respiratoryH Kan' | S. Andrews/Keio collection (48) |
| JW3818       | BW23113 ΔfimAHKan'        | National BioResource Project/Keio collection (48) |
| CAG25198     | MG1655 lacX74 lambda (pHP3-lacZ) nadB::Tn10 ΔrseA | C. Gross (49) |
| CAG45114     | MG1655 lacX74 lambda (pHP3-lacZ) | C. Gross (50) |
| CAG62192     | micACam'                  | C. Gross (26) |
| AAEC090      | MG1655 ΔlacZYA1 (SacB- Kan') | 28 |
| AAEC100      | AAEC090 (SacB-Kan')       | 28 |
| AAEC261A     | MG1655 ΔlacZYA fimB-lacZYA | 31 |
| BGEC043      | MG1655 ΔlacZYA ΔfimB (−457 [EcoO109I] to +209 [ClaI] relative to fimB ORF) Ω ( sacB-Kan') lacZYA-3' fimB | Our unpublished work |
| BGEC088      | MG1655 ΔlacZYA FimE-LacZ  | 6 |
| BGEC378      | MG1655 ΔlacZYA fimA'-R Nas III cleavage site-lacZYA fimE-am18 | 51 |
| BGEC905      | MG1655 ΔlacZYA FimB-LacZ  | 6 |
| KCEC840      | MG1655 ΔlacZYA ΔfimB (−1033 [ApaI] to +209 [ClaI] relative to fimB ORF) Ω ( sacB-Kan') lacZYA-3' fimB | 34 |
| KCEC1243     | BGEC905 ΔslyAHKan'        | 34 |
| KCEC1457     | BGEC905 Δ (ΔfimB 5' UTR −277 bp to −203 bp from fimB ORF) | This study |
| KCEC2862     | AAEC261A ΔslyAHKan'       | This study |
| KCEC3700     | BGEC905 ΔΔfimAHKan'       | This study |
| KCEC3858     | BGEC905 ΔΔfimB 5' UTR −196 bp to −20 bp from fimB ORF) | This study |
| KCEC3882     | BGEC905 ΔΔfimB 5' UTR −196 bp to −20 bp from fimB ORF) Ω (ΔfimH Kan') | This study |
| KCEC3890     | AAEC261A ΔΔfimH Kan'      | This study |
| KCEC4138     | BGEC905 micACam'          | This study |
| KCEC4176     | BGEC905 micACam' ΔΔfimH Kan' | This study |
| KCEC4198     | BGEC378 ΔΔfimH Kan'       | This study |
| KCEC4202     | KCEC2862 ΔslyA            | This study |
| KCEC4222     | KCEC4204 ΔfimH Kan'       | This study |
| KCEC4271     | BGEC905 ΔfimB (5'GGCGGTAGTo 5'CCGCTATCA) | This study |
| KCEC4275     | BGEC905 ΔfimB (5'GGCGGTAGTo 5'CCGCTATCA) | This study |
| KCEC4279     | BGEC905 ΔfimB (5'GGCGGTAGTo 5'CCGCTATCA) | This study |
| KCEC4326     | BGEC905 ΔΔfimB 5' UTR −196 bp to −58 bp from fimB ORF) | This study |
| KCEC4336     | BGEC905 ΔΔfimB 5' UTR −196 bp to −58 bp from fimB ORF) Ω (ΔfimH Kan') | This study |
| KCEC4364     | BGEC905 ΔΔfimB 5' UTR −277 bp to −203 bp from fimB ORF) | This study |
| KCEC4370     | AAEC261A ΔfimB (5'GGCGGTAGTo 5'CCGCTATCA) | This study |
| KCEC4372     | BGEC905 ΔΔfimH Kan' ΔlaczYAMtrfaH (from 161 bp upstream to 56 bp downstream of the rfaH ORF) | This study |
| KCEC4386     | BGEC908 ΔΔfimH Kan'       | This study |
| KCEC4412     | CAG45114 ΔΔfimH Kan'      | This study |
| KCEC4418     | CAG45114 nadB::Tn10 ΔrseA | This study |
| KCEC4420     | BGEC905 nadB::Tn10 ΔrseA  | This study |
| KCEC4454     | AAEC261A ΔΔfimH Kan'      | This study |
| KCEC4484     | BGEC905 micACam' nadB::Tn10 ΔrseA | This study |
| KCEC4534     | AAEC100 micA-lacZYA       | This study |
| KCEC4536     | KCEC4534 ΔΔfimH Kan'      | This study |
| KCEC4540     | KCEC4534 nadB::Tn10 ΔrseA  | This study |
agents were obtained from Sigma unless otherwise indicated. Liquid cultures were grown aerobically at 37°C, and culture densities were monitored spectrophotometrically at 420 or 600 nm.

**Analysis of fimB and fimE expression.** fimB and fimE expression was measured using a FimB-LacZ or FimE-LacZ translational fusion or fimB-lacZ transcriptional fusion situated in the chromosome at fim as described previously (6, 7, 31). H9252-Galactosidase assays were conducted as described by us previously (31), following growth in RD medium at 37°C with rapid aeration to an optical density at 600 nm (OD600) of approximately 0.2. Experiments were repeated at least twice, and the values shown in Miller units represent the mean of at least four samples with 95% confidence intervals included for each value.

**Determination of inversion frequencies.** Inversion of the fim switch was measured following growth in RD medium as described previously (32). Inversion frequencies were measured by inoculating 25 cultures with approximately 0.3 cells per tube. The ratio of on to off cells was estimated by plating at least five replicates onto lactose-MacConkey indicator medium after approximately 22 generations of growth at 37°C with rapid aeration.

**DNA manipulations.** DNA manipulations were carried out using standard protocols (33). Plasmid DNA was isolated using the miniprep or midiprep kit (Qiagen). Restriction enzymes and DNA ligase were purchased from either Promega or New England BioLabs. DNA sequencing was performed by Source BioScience, Nottingham, United Kingdom. Oligonucleotide synthesis was performed by MWG-Biotech AG or by Qiagen Operon, Germany. DNA was amplified by PCR using Pwo polymerase (Boehringer Mannheim) as described previously (25) or Q5 master mix (New England BioLabs). Oligonucleotides used in this study are listed in Table S1 in the supplemental material.

**RESULTS**

**RfaH is a positive regulator of fimB expression.** To determine if RfaH controls fimB expression, a ΔrfaH mutant of strain BGEC905 (MG1655 ΔlacZYA FimB-LacZ) was constructed by P1vir transduction. The level of β-galactosidase produced by the mutant was diminished by almost 3-fold relative to the wild type in this FimB-LacZ protein fusion background (Fig. 1). FimB recombination parallels fimB expression closely, and FimB recombination was also diminished around 9-fold in the rfaH mutant as anticipated (Fig. 2)(6, 7, 34).

In contrast to the FimB-LacZ fusion, expression of a comparable fimB-lacZYA operon fusion increased to a modest extent (~1.2-fold). Likewise, expression of a FimE-LacZ protein fusion also increased slightly (~1.2-fold; data not shown). As a control, the ΔrfaH-fimB mutant phenotype was complemented by an ectopic copy of the rfaH gene inserted into the chromosome at lac (data not shown). As an additional control, the effect of SlyA on the β-galactosidase produced by the two fusions was also measured (Fig. 1). SlyA activates fimB transcription by inhibiting H-NS repression, and its loss decreased expression of both fusions as expected (34). Thus, RfaH affects fimB expression mainly at the posttranscriptional level, to produce a net stimulatory effect on fimB expression.

**Identification of a region of the fimB 5’ UTR required for RfaH control.** In addition to its effects on transcription termination, RfaH stimulates translation initiation by binding to protein S10 of the 30S ribosomal subunit (22). This suggested to us that RfaH might activate fimB translation directly. Alternatively, we supposed that RfaH could activate fimB expression indirectly by
controlling the expression of posttranscriptional regulator instead.

Direct control by RfaH requires a cis-acting ops (operon polarity suppressor; consensus of 5’ R GGGCGTAGYNT) site downstream of the transcriptional start site, typically positioned far upstream of the translational start site (35, 36). fimB has a large (271-bp) 5’ UTR, and to screen for cis-active sequences required for RfaH control, three deletions were constructed in this region and transferred into the chromosome at fim to replace the wild-type regulatory region of the FimB-LacZ fusion (Fig. 3). The first deletion (Δ1) extends from immediately adjacent to the −10 region of the fimB promoter (−277 bp to −203 bp relative to the fimB open reading frame [ORF]) to an SphI restriction endonuclease site. The second (Δ2) and third (Δ3) deletions extend from the SphI site to −20 bp and −20 bp relative to the fimB ORF, respectively. The best match (5’ AAGGGA) to the consensus Shine-Dalgarno sequence (5’ AGGAGG) extends from −12 bp to −7 bp relative to the fimB ORF.

All of the deletion mutations increased the expression of the FimB-LacZ fusion to a greater or lesser extent, suggesting that the long intergenic region of fimB has a detrimental effect on fimB expression overall (Fig. 4). While the Δ1 mutation increased the response to RfaH considerably (3-fold in the wild type to 7-fold in the Δ1 mutant), the Δ2 mutation decreased the response to RfaH to 2-fold and the Δ3 mutation eliminated it almost entirely.

**Effect of RfaH on MicA inhibition of fimB expression.** The σ54-controlled regulatory sRNA MicA inhibits fimB expression (26). The mRNA binding target for MicA lies immediately upstream of the fimB Shine-Dalgarno sequence (extending from −9 to −46) and is thus conserved in its entirety in the Δ2 mutation but absent from the Δ3 mutation (Fig. 3). These observations suggested to us that RfaH might activate fimB expression by somehow preventing MicA inhibition. Although MicA surprisingly had a net stimulatory effect on fimB expression in the wild-type background, mutation of micA nevertheless suppressed the stimulatory effect of RfaH on fimB expression entirely (Fig. 5). As a control, the effect of RseA on fimB expression was also tested. RseA prevents induction of the σ54 regulon by sequestering the sigma factor to the inner membrane (37). As expected, fimB expression was inhibited strongly in the rseA mutant background and this effect was also suppressed in an rseA micA double mutant. The results of these experiments thus support the conclusion that RfaH somehow prevents MicA inhibition of fimB expression.

**Loss of RfaH induces micA and rpoHP3 expression.** The results described above suggested that RfaH might exert an indirect effect on fimB expression by controlling micA expression. To test this hypothesis, the effect of both RfaH and RseA on expression of a micA-lacZ transcriptional fusion was determined. The results of this experiment (Fig. 6) demonstrate that loss of RfaH results in the induction of micA transcription, albeit to a lesser extent than that observed in the rseA mutant background (>5-fold versus >13-fold).

We supposed either that RfaH could activate micA expression directly or that, more likely, its loss leads to induction of the σ54 regulon in general. To distinguish between these possibilities, the effect of RfaH on expression of the σ54-specific rpoHP3 promoter (38) was also tested (Fig. 7). As expected, expression of the rpoHP3-lacZ construct was induced significantly by mutation of rseA (27-fold). Mutation of rfaH had a smaller (10-fold) effect, consistent with the more modest effect of RfaH on both fimB and micA expression.

Notwithstanding the results described above, it still seemed possible that RfaH might also exert a direct effect on fimB expression by binding to an ops-like element in the fimB 5’ UTR. This seemed plausible because the Δ2 mutation, which removes a significant part of the 5’ UTR, diminished the effect of RfaH on fimB expression (Fig. 4). Moreover, a search of the 5’ UTR of fimB highlighted a potential ops-like site (5’ TGGCGTTTGTAT; non-ops-matching bases underlined) positioned around 180 bp downstream of the fimB translational start (Fig. 3). This ops-like sequence (here termed OLE for ops-like element) lies 8 bp downstream of the SphI site present in the fimB 5’ UTR and hence is deleted in both the Δ2 and Δ3 mutants. However, the effect of rseA on fimB expression was also decreased from >10-fold in the wild-type background to <4-fold in the Δ2 mutant background, suggesting that the Δ2 mutation diminishes the effect of MicA on fimB expression (data not shown). Moreover, while mutation of OLE from 5’ TGGCGTTTGTAT to TCCGCTATCAAT did decrease fimB expression >8-fold, this effect did not require RfaH (data not shown). Furthermore, the OLE mutation also decreased the expression of the fimB-lacZ transcription fusion (data not shown), which mutation of rfaH does not (Fig. 1). We thus conclude that loss of RfaH leads to induction of σ54, and hence micA, and that this effect accounts for most, if not all, of the stimulatory effect of RfaH on fimB expression.

The effect of procaine on fimB expression and Fim recombination. σ54, and hence MicA, is induced by procaine and by
ethanol (39, 40). As noted above, although MicA had a net stimulatory effect on fimB expression under noninducing conditions (Fig. 5), inclusion of increasing amounts of procaine (Fig. 8) or of ethanol (data not shown) led to a dose-dependent decrease in fimB expression as expected. FimB recombination was also inhibited by procaine as anticipated (Fig. 9). Procaine induces H9268E by activating the EnvZ-OmpR two-component regulatory system (39). In agreement with this assertion, the effect of procaine on fimB expression was also diminished in an ompR mutant background. Moreover, the level of fimB expression in a micA ompR double mutant was indistinguishable from that in the ompR single mutant background (data not shown).

Surprisingly, inhibition of fimB expression by both procaine (Fig. 8) and ethanol (data not shown) was still apparent, albeit to a decreased extent, in the micA mutant background. Unexpectedly, RfaH actually inhibited fimB expression in the absence of MicA under σ52-inducing conditions. Further work demonstrated that procaine also inhibits expression of the fimB-lacZ transcriptional fusion and that this effect requires RfaH (Fig. 10) but not OmpR (data not shown).

The results described above suggest that procaine might trigger an alternative envelope stress response pathway that is also controlled by RfaH. According to this scenario, we supposed that both procaine and RfaH activate the pathway and that the cognate regulator of the system inhibits fimB transcription. It has been shown previously that fimB transcription is controlled by the response regulator RcsB that forms part of the Rcs phosphorelay system, a regulatory pathway that is also responsive to envelope stress (41). However, it was found that fimB transcription was unaffected by RcsB under the growth conditions used in this study and that the effect of procaine on the expression of the fimB-lacZ transcriptional fusion remained intact in this mutant background (data not shown). In contrast, the response of fimB transcription to procaine was found to be dependent upon SlyA (Fig. 10). Furthermore, the inhibitory effect of RfaH on fimB transcription was enhanced in the slyA mutant background.

**FIG 5** The effects of micA, rfaH, and micA rfaH double mutations on the β-galactosidase produced by a FimB-LacZ fusion. The wild-type (wt) and mutant strains indicated were grown and processed as described in Materials and Methods, except that the growth medium used contained 1 mM nicotinic acid to allow growth of the rseA mutants which contain a linked nadB::Tn10 mutation.

**FIG 6** The effects of rfaH and rseA on the β-galactosidase produced by a micA-lacZ fusion. The wild-type (wt) and mutant strains indicated were grown and processed as described in Materials and Methods, except that the growth medium used contained 1 mM nicotinic acid to allow growth of the rseA mutants which contain a linked nadB::Tn10 mutation.

**FIG 7** The effect of rfaH and rseA on the β-galactosidase produced by a rpoH:P3-lacZ fusion. The strains indicated were grown and processed as described in Materials and Methods, except that the growth medium used contained 1 mM nicotinic acid to allow growth of the rseA mutants which contain a linked nadB::Tn10 mutation.
The results presented above are consistent with our model that RfaH activates fimB expression by preventing MicA inhibition. However, they also suggest that RfaH can, at least in the presence of procaine, somehow inhibit fimB transcription by a mechanism that involves neither OmpR nor RcsB but which does require SlyA. These results highlight the complexity of the RfaH regulatory circuit that controls fimB expression.

DISCUSSION

RfaH-binding ops elements are characteristically found in long 5′ UTRs, far upstream of ORFs. Moreover, UPEC mutants lacking rfaH are attenuated for virulence in an ascending mouse model of urinary tract infection (21). Since fimB has a relatively large (271-bp) 5′ UTR and type 1 fimbriation is a virulence factor in the mouse model, we considered it possible that RfaH is an activator of fimB expression. Here, we demonstrate that RfaH does indeed enhance fimB expression, but further analysis reveals that this effect is indirect.

In support of the hypothesis that RfaH enhances fimB expression, it was found in an initial experiment that expression of a FimB-LacZ protein fusion was diminished around 3-fold in an rfaH deletion mutant. Surprisingly, however, expression of a comparable fimB-lacZ transcriptional fusion was elevated slightly in the absence of RfaH, indicating that RfaH activates fimB expression selectively at the posttranscriptional level. Moreover, deletion analysis of the fimB 5′ UTR indicated that, rather than requiring sequences far upstream of the fimB ORF, RfaH control is dependent upon sequences close to the ribosome binding site. Further work demonstrates that RfaH activates fimB expression indirectly by controlling induction of σH and hence the sRNA MicA (Fig. 11).

In addition to controlling the expression of a number of virulence factors, RfaH enhances expression of the waaQ operon required for LPS core biosynthesis (15, 16). Alterations to LPS core biosynthesis, apparently by inducing misfolding of outer membrane proteins, can also induce σE (42). While not proven here, we postulate that induction of σE in the rfaH mutant reflects the involvement of RfaH in LPS biosynthesis (Fig. 11). We note that the increased autoaggregation factor antigen 43 (Ag43)–enhanced biofilm formation observed in an rfaH mutant background was also attributed in part to changes in LPS biosynthesis (17).

The effect of procaine on σE induction, and hence mica expression, was reported previously to be dependent upon OmpR (39). Our results agree with this since the effect of procaine on fimB expression was diminished in an ompR mutant and the level of fimB expression in the micA ompR double mutant was indistinguishable from that of the ompR single mutant across the range of procaine levels (0 to 20 mM) tested (data not shown). Surprisingly, the residual effect of procaine on fimB expression in a micA mutant is largely dependent on RfaH (Fig. 8). Further work shows that procaine inhibits expression of the fimB-lacZ transcriptional fusion as well and that this effect requires RfaH (Fig. 10) but not OmpR (data not shown). We suppose that this additional effect of procaine involves an alternative stress-response pathway and an unidentified transcriptional repressor (Rx) (Fig. 11). RfaH has also been shown somehow to inhibit transcription of flu, which encodes Ag43 (17). This effect, which is due neither to changes in flu phase variation per se nor to altered control by known regulator OxyR or Dam, suggests that fimB and flu transcription may both be repressed by Rx.

The effect of RfaH and procaine on fimB transcription is not dependent upon OLE (data not shown). Moreover, we have also
ruled out involvement of the Rcs phosphorelay system, which has been reported to control fimB expression and which is also responsive to envelope stress (data not shown) (41). On the other hand, the effect of procaine on fimB transcription requires SlyA (Fig. 10). Although these results are open to interpretation, we favor a model in which RfaH is necessary for expression of the alternative stress-response pathway while loss of SlyA leads to its constitutive activation (Fig. 11). We suppose that SlyA activates the expression of an additional unidentified factor (Fx) that somehow alters the signaling pathway to make it responsive to procaine (Fig. 11).

Type 1 fimbriae, which are anchored in the bacterial outer membrane, facilitate the delivery of LPS to the TLR4 signaling pathway in CD14-negative epithelial cells (10). Furthermore, in contrast to phase-locked-off bacteria, MicA inhibits fimB expression in phase-locked-on fimbriate cells even in the absence of exogenous inducers of σE (our unpublished data). This suggests to us that fimbrial biosynthesis itself imposes significant stress on the outer membrane, as has been reported for other outer membrane proteins (38). We thus propose that suppressing fimB expression, and hence type 1 fimbriation, in response to the integrity of the bacterial outer membrane is an adaptation that enhances bacterial survival both by diminishing envelope stress and by limiting the host inflammatory response. The fact that RybB, a second σE-dependent sRNA, inhibits fimA expression would provide an additional mechanism to limit fimbrial expression in phase-on bacteria (26). Indeed, we suppose that this explains why nonfimbriate cells are produced even when fimS is locked in the off phase, or when fimS is replaced with the isopropyl-β-D-galactopyranoside (IPTG)-inducible tac promoter (43). Mutation of surA also induces σE (44), and as expected, we have found that fimB expression was also decreased in a surA mutant (data not shown). While SurA enhances the correct localization of the fim usher (FimD) to the outer membrane, our results indicate that decreased fimB expression, and hence off-to-on inversion of fimS, as well as induction of rybB, also contributed to diminished type 1 fimbriation observed in a surA mutant (45).

A BLAST search of the nucleotides of the fimB mRNA predicted to bind to MicA demonstrates that these sequences are perfectly conserved in all of the E. coli strains for which DNA sequence data are available. Thus, the regulation of fimB expression by MicA, and its control by factors such as RfaH and SurA, is likely to have general significance for the control of type 1 fimbriation in most, if not all, E. coli strains. Further work will determine how RfaH controls σE, as well as the response of fimB transcription to procaine, and the full extent of these control pathways on type 1 fimbriation in commensal and pathogenic strains alike.

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