Research Article

Antagonistic Roles for GcvA and GcvB in hdeAB Expression in Escherichia coli

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In E. coli, the periplasmic proteins HdeA and HdeB have chaperone-like functions, suppressing aggregation of periplasmic proteins under acidic conditions. A microarray analysis of RNA isolated from an E. coli wild type and a ΔgcvB strain grown to mid-log phase in Luria-Bertani broth indicated the hdeAB operon, encoding the HdeA and HdeB proteins, is regulated by the sRNA GcvB. We wanted to verify that GcvB and its coregulator Hfq play a role in regulation of the hdeAB operon. In this study, we show that GcvB positively regulates hdeA::lacZ and hdeB::lacZ translational fusions in cells grown in Luria-Bertani broth and in glucose minimal media + glycine. Activation also requires the Hfq protein. Although many sRNAs dependent on Hfq regulate by an antisense mechanism, GcvB regulates hdeAB either directly or indirectly at the level of transcription. GcvA, the activator of gcvB, negatively regulates hdeAB at the level of transcription. Although expression of gcvB is dependent on GcvA, activation of hdeAB by GcvB occurs independently of GcvA’s ability to repress the operon. Cell survival and growth at low pH are consistent with GcvA negatively regulating and GcvB positively regulating the hdeAB operon.

1. Introduction

Acid resistance is important for the ability of enteric bacteria to survive the low pH environment encountered in the gastrointestinal tract of mammalian hosts and other natural environments [1]. Enteric bacteria have five systems of acid resistance [2–7]. The first system, AR1, is least understood. When cells are grown in LB at pH 5 to stationary phase, they survive dilution into minimal medium at pH 2.5, which kills cells grown at pH 8. The stationary phase sigma factor RpoS and cyclic-AMP receptor protein are required to develop acid tolerance [2, 6]. The other four systems, AR2 AR3, AR4, and AR5, are decarboxylate/antiporter-dependent acid resistance systems that require glutamate, arginine, lysine, and ornithine, respectively [2, 4–10]. Additional acid protection comes from the periplasmic proteins HdeA and HdeB that have chaperone-like functions, suppressing aggregation of periplasmic proteins under extreme acidic conditions [11–13]. Both hdeA and hdeB mutants show reduced viability upon acid stress and HdeA/HdeB expressing plasmids restore viability close to wild type, suggesting both proteins are necessary for protection of the bacterial periplasm against acid stress [14]. Regulation of the hdeAB operon is complex. The hdeAB operon in E. coli is acid inducible and regulation involves GadE, RpoD, RpoS, H-NS, MarA, and several other regulators [6, 7, 15–18].

The E. coli gcvB gene encodes a sRNA of 206 nucleotides [19]. Expression of gcvB is activated by the GcvA protein when cellular glycine is high and repressed by GcvA and GcvR when glycine is limiting [19]. In both E. coli and Salmonella enterica serovar Typhimurium, GcvB regulates genes involved in the transport of small peptides and polar and branched amino acids [19–24]. Recently, it was shown GcvB enhances the ability of E. coli to survive low pH by upregulating RpoS [25]. In addition, microarray data suggested the hdeAB operon is positively regulated by GcvB [22]. Results from this study establish a role for GcvA in repressing the hdeAB operon and GcvB in activating the operon. Hfq, an RNA chaperone required for GcvB regulation of known target genes [20, 22, 23, 26], is also required for activation. However, the results suggest GcvB and Hfq do not function as an antisense RNA system to upregulate hdeAB translation,
but act at the level of transcription. The results also suggest GcvA, the activator for gcvB, negatively regulates hdeAB at the level of transcription.

2. Materials and Methods

2.1. Bacterial Strains, Plasmids, and Phage. The E. coli strains, plasmids, and phage used are listed in Table 1 or described in the text.

2.2. Construction of Recombinant Phages and Plasmids. The λhdeA::lacZ translational fusion was constructed by PCR synthesis of a DNA fragment using an upstream primer with an EcoRI site that hybridized to DNA beginning 223 bps upstream of the hdeA transcription start site and a downstream primer with a SmaI site that hybridized to DNA beginning at codon 7 within the hdeA gene. The PCR amplified DNA fragment was digested with EcoRI and cloned into the EcoRI site of phage λgt2 [30], generating λhdeA::lacZ. A λhdeB::lacZ fusion was constructed using the same upstream primer and a downstream primer with a SmaI site at the 5′ end of the lacZYA genes in pMCI1403 (Figure 1(a)). The cloned sequence was verified by DNA sequence analysis at the DNA Core Facility of the University of Iowa. The plasmid was designated phdeA::lacZ. A 5,574 bp EcoRI-MfeI fragment from phdeA::lacZ carrying the hdeA::lacZYA fusion was then ligated into the EcoRI site of phage λgt2 [30], generating λhdeA::lacZ. A λhdeB::lacZ fusion was constructed using the same upstream primer and a downstream primer with a SmaI site that hybridized to DNA beginning at codon 9 within the hdeB gene. The 757 bp EcoRI-Smal fragment was then used as described above, generating plasmid phdB::lacZ and phλhdeB::lacZ (not shown). A λhdeA::lacZ transcriptional fusion was constructed using the same upstream primer and a downstream primer with a HindIII site and that hybridized to DNA at bp −36 relative to the hdeA translation start site (Figure 1(a)). Following digestion with EcoRI and HindIII, the fragment was ligated into the EcoRI and HindIII sites of plasmid pGCVBλlacZ +50 [19], replacing the gcvB fragment with the hdeA fragment, generating plasmid phdeA−36::lacZ. The cloned sequence was verified by DNA sequence analysis. A 5,538 bp EcoRI-MfeI fragment from phdeA−36::lacZ carrying the hdeA−36::lacZYA fusion was then ligated into the EcoRI site of phage λgt2 [30], generating λhdeA−36::lacZ. The 3 fusion phages were used to lysogenize E. coli host strains as described [38]. Each lysogen was tested to ensure it was a single-copy of the λ chromosome by infection with λcI90c17 [39]. All lysogens were grown at 30°C in LB with sodium chloride from 50–150 mg/L. T4 DNA ligase was from Roche Diagnostics (Indianapolis, IN). Reactions were as described by the manufacturers.

2.3. Media. The complex medium used was LB [41]. Agar was added at 1.5% (w/v) to make solid media. The minimal medium used was the salts of Vogel and Bonner [42] supplemented with 0.4% (w/v) glucose (GM). Ampicillin was added at 50 and 150 μg mL−1 when strains carried single-copy and multicopy plasmids, respectively. Other supplements were added at the following concentrations (μg mL−1): phenylalanine, 50; glycine, 300; thiamine, 1; TC, 10; CM, 20; X-gal, 40.

2.4. DNA Manipulation. Plasmid DNA was isolated using a QiAprep Spin Miniprep Kit (Qiagen, Santa Clara, CA). Vent DNA polymerase and restriction enzymes were from New England Biolabs, Inc. (Beverly, MA). T4 DNA ligase was from Roche Diagnostics (Indianapolis, IN). Reactions were as described by the manufacturers.

2.5. Enzyme Assay. β-galactosidase assays were performed on mid-log phase cells (OD600 ∼ 0.5) using the chloroform/SDS lysis procedure [41]. Results are the averages of two or more assays with each sample done in triplicate.

2.6. Acid Sensitivity Assay. WT, an isogenic ΔgcvA strain and the two strains transformed with either plasmid pgcvB3+ (constitutively produces GcvB), pgcvA3+ or pgcvA3+ gcvB3+ were grown for 24 hr at 30°C in LB and then tested for acid resistance by dilution into LB at pH 2.0. Samples of 0.2 mL were taken at 0, 1, 2, and 4 hr and diluted in 2 mL of LB at pH 7. The final pH of the diluted cultures was ∼7.0. Cell viability was determined by plate counts. Percent survival is the titer of colony forming units of acid-tested cells compared to the zero-time point (Figure 2).

2.7. Transductions. The gcvB gene is linked to the argA gene and hfq is linked to the cycA gene, with predicted phage P1 cotransduction frequencies of ∼78% and ∼67%, respectively. P1clr phase prepared on GS854 (argA1::Tn10) was used to transduce ΔgcvB::ΔCmsλhdeA::lacZ to TC R and transductants scored on CM versus TC plates. A TC R CM3 transductant was purified. P1clr prepared on GS776 (cycA::Tn10) was used to transduce Δhfq1::ΔCmsλhdeA::lacZ to TC R resistance and transductants scored on CM versus TC plates. A TC R CM3 transductant was purified.
3. Results and Discussion

3.1. GcvA/GcvB Role in Acid Sensitivity. Microarray data suggested the hdeA and hdeB mRNAs are 1.9- and 2.7-fold higher in WT than a ΔgcvB strain grown in LB, respectively [22]. These genes were not reported to be regulated by GcvB in that study because they fell below the 3-fold cut-off level used for GcvB-regulated genes. Since HdeA and HdeB are necessary for protection of the bacterial periplasm against acid stress [14, 17], we tested if GcvB plays a role in cellular acid resistance. WT and an isogenic ΔgcvA strain grown for 24 hr at 30°C were still in log phase. In E. coli and Salmonella grown in LB, GcvB was only detected through early stationary phase, with the highest levels observed at the mid-exponential phase [20, 26]. Thus, GcvB regulation of target genes involved in acid resistance is likely during log phase and if GcvB plays a role in stationary phase, it is its absence that is important for acid resistance [25]. Our failure to observe a significant effect on acid resistance is possibly due to the assay conditions. We tested for acid resistance after 24 hours of growth in LB, whereas in the earlier study acid resistance was tested after 5 hr of growth in LB [25]. Although the precise stage of growth was not stated in the earlier study, it is possible cells were still in log phase. In E. coli and Salmonella grown in LB, GcvB was only detected through early stationary phase, with the highest levels observed at the mid-exponential phase [20, 26]. Thus, GcvB regulation of target genes involved in acid resistance is likely during log phase and if GcvB plays a role in stationary phase, it is its absence that is important for allowing an appropriate regulatory response.

3.2. Effects of GcvB on λhdeA::lacZ Expression in LB Grown Cells. Although GcvB had no effect in the acid sensitivity assay, we made and tested expression of λhdeA::lacZ and λhdeB::lacZ translational fusions. Expression of the hdeA::lacZ fusion was 2.7- and 4-fold higher in WT grown in LB compared to ΔgcvB and Δhfq strains (Figure 3(a), compare lanes 1, 2 and 3). Activation was partially restored in the ΔgcvB[pGcvB+] and Δhfq[plfhy+] complemented strains (Figure 3(a), compare lanes 2 and 4 and lanes 3 and 5). It is unknown why the plasmids fail to fully complement the ΔgcvB and Δhfq mutations. Nevertheless, the results agree with microarray data and suggest GcvB and Hfq positively regulate hdeA::lacZ.

Table 1: Strains, plasmids, and phage.

<table>
<thead>
<tr>
<th>Strain, plasmid, or phage</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS162</td>
<td>WT</td>
<td>This lab</td>
</tr>
<tr>
<td>GS776</td>
<td>cycA30::Tn10</td>
<td>This lab</td>
</tr>
<tr>
<td>GS854</td>
<td>argA81::Tn10</td>
<td></td>
</tr>
<tr>
<td>GS998</td>
<td>GS162 gcvA</td>
<td>[27, 28]</td>
</tr>
<tr>
<td>GS1132</td>
<td>GS162 Δ(gcvA/gcvB)::aadA (referred to as ΔgcvAB)</td>
<td>[19]</td>
</tr>
<tr>
<td>GS1144</td>
<td>GS162 ΔgcvB::OCMR (referred to as ΔgcvB)</td>
<td>[21]</td>
</tr>
<tr>
<td>GS1148</td>
<td>GS162 hfq-1::OCMR (referred to as Δhfq)</td>
<td>[23]</td>
</tr>
</tbody>
</table>

Plasmid

| pGS554 | Single-copy vector + constitutive gcvB (pgcvB2+) | [19] |
| pGS571 | Multicopy vector + WT gcvB (pgcvB3+) | [29] |
| pGS594 | Single-copy vector + WT gcvB (pgcvB+) | This lab |
| pGS609 | Multi-copy vector + WT hfq (phfq+) | [23] |
| pGS611 | Multi-copy vector + WT gcvA (pgcvA3+) | This study |
| pGS624 | Multi-copy vector + WT gcvA gcvB (pgcvA3+gcvB3+) | This study |

Phage

| λgt2 | λ cloning vector; cI857 repressor | [30] |
| λhdeA::lacZ | λ vector carrying WT hdeA::lacZ fusion | This study |
| λhdeB::lacZ | λ vector carrying WT hdeB::lacZ fusion | This study |
| λhdeA-36::lacZ | λ vector carrying hdeA-36::lacZ transcripational fusion | This study |
| λPbad::hdeA::lacZ | λ vector carrying hdeA::lacZ fusion under control of the Pbad promoter | This study |

*All strains also carry the pheA905 thi araD129 rpsL150 relA1 deoC1 flbB5301 ptsF25 rbsR mutations.
3.3. Reduced hdeA::lacZ Expression in ΔgcvB and Δhfq Strains
Is due to the Absence of GcvB and Hfq. Due to the failure of pgcBV+ and phfq+ to fully complement the gcvB and hfq mutations (Figure 3), we wanted to verify the reduced levels of hdeA::lacZ expression are due to the absence of GcvB and Hfq. We transduced the ΔgcvB and Δhfq lysogens with WT alleles using linked Tn10 markers. The gcvB+ and hfq+ transductants showed about the same levels of expression as the WT lysogen (Figure 3(a)), compare lanes 1, 12 and 13). Thus, despite the failure of pgcBV+ and phfq+ to fully complement, the results support the reduced levels of expression are due to the absence of GcvB and Hfq.

3.4. Effects of GcvA on λhdeA::lacZ Expression in LB Grown Cells. The acid sensitivity assay showed GcvA plays a role in acid resistance (Figure 2). In addition, putative GcvA binding sites can be identified in the hdeA promoter region (Figure 1(a)). Thus, we tested the effects of a spontaneous gcvA mutation in strain GS1198 (which is phenotypically GcvB− [19]), on hdeA::lacZ expression. Expression of hdeA::lacZ was ∼1.5-fold higher in WT than in the gcvA mutant (Figure 3, lanes 1 and 6). However, expression was 2-fold higher in the gcvA lysogen than in the ΔgcvB lysogen (Figure 3(a), compare lanes 2 and 6). The results could be explained if GcvA, in addition to activating expression of gcvB, which encodes a positive regulator for hdeA, also has a negative role to keep HdeAB levels low. The intermediate level of expression would result from the absence of GcvB to upregulate the hdeA::lacZ fusion and the absence of GcvA to negatively regulate the fusion. To test this hypothesis, we transformed the gcvA mutant with multi-copy pgcBV+. In the gcvA[pgcBV+] lysogen, one would expect high GcvA levels, but GcvB would also be produced. Expression of hdeA::lacZ was 2.5-fold higher in WT than in the gcvA[pgcBV+] transformant (Figure 3(a), compare lanes 1 and 8). In addition, hdeA::lacZ expression was reduced 2-fold
compared to the nontransformed gcvA strain (Figure 3(a), lanes 6 and 8). We then transformed the ΔgcvAB lysogen with pgcvaA3+. In the ΔgcvAB[pgcvaA3+] lysogen, there would be high GcvA levels and no GcvB, and repression of hdeA::lacZ should be greatest. In the ΔgcvAB[pgcvaA3+] lysogen there was a 5.8-fold reduction of hdeA::lacZ expression compared to WT and a 2.4-fold reduction compared to the ΔgcvAB lysogen (Figure 3(a), compare lanes 1, 7 and 9). The results support a role for GcvA in negatively regulating hdeA::lacZ expression.

3.5. GcvB Positively Regulates hdeA::lacZ Independent of GcvA. If GcvA plays a negative role in hdeA::lacZ expression, GcvB could function to prevent the GcvA effect. Alternatively, GcvB could function independent of GcvA to activate hdeA::lacZ. To test these two possibilities, we transformed the ΔgcvABΔhdeA::lacZ lysogen with pgcvB3+, which makes GcvB constitutively [19]. If GcvB’s role is to block GcvA’s ability to repress hdeA::lacZ expression, we hypothesized there would be no effect of GcvB in a ΔgcvABΔhdeA::lacZ lysogen without GcvA. Alternatively, if GcvB positively regulates hdeA::lacZ, we hypothesized expression of gcvB would increase hdeA::lacZ expression. In the ΔgcvAB[pgcvaB3+] lysogen, hdeA::lacZ expression increased 1.7-fold compared to the non-transformed lysogen, almost to the WT level (Figure 3(a), compare lanes 1, 7 and 10). We also transformed the ΔgcvABΔhdeA::lacZ lysogen with pgcvaA3+ gcvB3+, which overproduces both GcvA and GcvB. Repression of hdeA::lacZ was restored, but not as low as in the pgcvaA3+ transformant (Figure 3(a), compare lanes 9 and 11). It is likely that the high GcvB levels partially negate the effect of high GcvA levels. The results suggest GcvB plays a role in activating hdeA::lacZ independent of GcvA.

3.6. Effect of GcvA, GcvB, and Hfq on hdeA::lacZ Expression. In E. coli, GcvB represses dppA::lacZ, oppA::phoA, cyaA::lacZ, and sttT::lacZ fusions when cells are grown in LB, but does not significantly repress these fusions when grown in GM + glycine [19, 22, 23]. However, gcvB is differentially regulated over a 25-fold range in GM supplemented with inosine versus glycine [19]. We hypothesize some genes respond to GcvB levels in GM media. Microarray data suggested the hdeA mRNA is 1.6-fold higher in WT than a ΔgcvB strain grown in GM + glycine [22]. In GM + glycine, hdeA::lacZ expression was significantly higher than for cells grown in LB (Figure 3, compare a and b). In addition, although there are small differences in fold regulation for individual strains, there was a similar regulatory pattern in GM + glycine as observed in LB. The results suggest GcvB positively regulates hdeA in LB and GM + glycine. The results are important since they confirm GcvB does regulate in GM + glycine. In addition, acid resistance mechanisms are most active in the stationary phase in rich media [6, 7, 44, 45]. Our results suggest GcvA and GcvB could play important roles in acid resistance during the log phase of growth in both rich and minimal medium.

3.7. Effect of GcvA, GcvB, and Hfq on hdeB::lacZ Expression. The hdeB gene is the second gene in the hdeAB operon. We tested if hdeB is regulated in a manner similar to the hdeA gene. There were small differences in the levels of hdeB::lacZ expression in response to GcvB, GcvA, and Hfq compared to hdeA::lacZ in both LB and GM + glycine (compare Figures 3(a) and 3(b) with Figures 3(c) and 3(d)). Qualitatively, however, the hdeB::lacZ fusion showed essentially an identical pattern of expression compared to the hdeA::lacZ fusion, suggesting both genes of the operon are regulated in a similar manner by GcvA, GcvB, and Hfq.

It is worth noting that Δhfq lysogens consistently showed lower levels of hdeA::lacZ and hdeB::lacZ expression than ΔgcvaB lysogens in both LB and GM + glycine (Figure 3). Two other sRNAs, DsrA, and GadY, are known to play roles in regulation of acid-resistance genes [46, 47]. Since both sRNAs require Hfq, it is not surprising the absence of Hfq has a more dramatic effect on hdeAB expression than the absence of GcvA.

3.8. High Levels of GcvA, GcvB, and Hfq in WT Alters hdeA::lacZ and hdeB::lacZ Expression. To verify GcvA negatively regulates and GcvB and Hfq positively regulate the hdeAB operon, we transformed WT::hdeA::lacZ, and WT::hdeB::lacZ lysogens with plasmids carrying gcvA, gcvB, both gcva + gcvB, or hfq. We hypothesized high GcvB and Hfq would increase expression and high GcvA would repress expression. The lysogens were grown in LB and assayed for β-galactosidase. The presence of pgcvB3+ resulted in a small increase in hdeA::lacZ expression and about a 2-fold increase in hdeB::lacZ (Figures 4(a) and 4(b), lines 1 and 2).
The presence of p\textit{hfq} resulted in a 2-fold increase in both \textit{hdeA}:\textit{lacZ} and \textit{hdeB}:\textit{lacZ} expression (Figures 4(a) and 4(b), line 3). The presence of p\textit{gcvA} resulted in a 3.5-fold and a 2.2-fold reduction in \textit{hdeA}:\textit{lacZ} and \textit{hdeB}:\textit{lacZ} expression, respectively (Figures 4(a) and 4(b), line 4). The presence of plasmid p\textit{gcvA} \textit{gcvB} reduced \textit{hdeA}:\textit{lacZ} and \textit{hdeB}:\textit{lacZ} expression, but not to the levels of the p\textit{gcvA} plasmid (Figures 4(a) and 4(b), line 5), suggesting high GcvB antagonize the GcvA effect.

The lysogens were also grown in GM + glycine. The pattern of regulation was similar to the LB grown lysogens with one exception. The p\textit{gcvB} transformant did not show increased expression of \textit{hdeB}:\textit{lacZ} as in LB (Figure 4(b), compare lines 1 and 2 with lines 6 and 7). It is possible that in WT grown in GM + glycine GcvB is already in excess for regulation. Nevertheless, the results are in agreement with GcvB and Hfq positively regulating the \textit{hdeAB} operon and GcvA negatively regulating the operon.

3.9. GcvA, GcvB, and Hfq Regulate \textit{hdeA}:\textit{lacZ} at the Level of Transcription. GcvA binds DNA and functions to either activate or repress transcription [27, 35, 48], whereas sRNAs that require Hfq usually regulate posttranscriptionally [19, 21–23]. To determine at what step in regulation of \textit{hdeA} GcvA,
GcvB and Hfq function, we constructed a λP_BAD::hdeA::lacZ fusion where transcription from the P_BAD promoter begins at the +1 start site of the hdeA gene (Figure 1(b)). We initially lysogenized a WT strain with the fusion, the lysogen was grown in LB + arabinose (0.0 to 0.2% concentrations) and assayed for β-galactosidase. There was a 379-fold induction (2.3 units versus 872 units of activity) at 0.0% and 0.05% arabinose, respectively. This is similar to the level observed from the λhdeA::lacZ lysogen grown in LB (Figure 3) and confirmed the fusion is inducible by arabinose. We then lysogenized WT, ΔgcvB, ΔgcvAB, and Δhfq strains. The WT lysogen was also transformed with the plasmids indicated in Figure 5(a). The strains were grown in LB + 0.05% arabinose and assayed for β-galactosidase. If GcvA, GcvB, and Hfq regulate at the transcriptional level, we expected they would no longer have an effect on the P_BAD::hdeA::lacZ fusion. Alternatively, if any of the factors regulates post-transcriptionally, we expected it would still regulate the fusion, as the mRNA is identical to the WT λhdeA::lacZ mRNA transcript. There was no significant difference in P_BAD::hdeA::lacZ expression in the WT, WT[pGcVA^3+] and WT[pGcVA^3+gcvB^3+] transformants (Figure 5(a), compare
show GcvB and Hfq function during log phase to positively regulate hdeAB at the transcriptional level, counterbalancing the negative effect of GcvA on downregulating these genes. GcvB is known to bind Hfq [49]. It is possible GcvB binds to and sequesters Hfq during exponential growth, and the effects observed are due to decreased levels of Hfq to alter regulation of genes such as rpoS or the activity of sRNAs such as DsrA and GadY that play roles in acid resistance. Additional studies will verify if GcvA directly binds the hdeAB promoter region and how GcvB and Hfq activate the operon.

3.10. Effect of pH on gcvB Expression. Our results suggest GcvB plays a role in acid resistance during log phase of growth in rich and minimal media. Therefore, we tested if pH plays a role in regulating gcvB expression. A WTΔgcvB: lacZ fusion was grown to mid-log phase in LB at different pH values from 5.0 to 9.0 and assayed for β-galactosidase. There was no significant effect from pH 7 to pH 9 on gcvB: lacZ expression (Figure 6). However, there was a 3-fold increase as the pH was lowered from pH 7 to pH 5 (Figure 6). Since GcvB activates hdeAB, an increase in gcvB expression at low pH is likely to play a role in final HdeAB levels and in controlling acid resistance.

3.11. Effect of GcvA and GcvB on Cell Growth at Low pH. We carried out studies to show the effects of high GcvA and GcvB levels on growth at low pH. In a ΔgcvAB strain transformation with pgcvA<sup>3+</sup> or pgcvAB<sup>3+</sup>gcvB<sup>3+</sup> did not significantly alter generation times (GTs) in LB at pH 7 (Table 2). At pH 4.5, GTs of both the WT and ΔgcvB strains were significantly increased (Table 2, compare rows 1 and 2, pH 7.0 versus pH 4.5). In addition, in the WT[pgcvA<sup>3+</sup>] strain, with high GcvA and low GcvB, there was a significant increase in the GT compared to the non-transformed WT strain (Table 2, compare rows 1 and 3, pH 4.5). In the gcvAB[pgcvA<sup>3+</sup>] transformant, with high GcvA and no GcvB, there was an additional increase in the GT (Table 2, compare rows 2 and 4, pH 4.5 column). In the WT[pgcvA<sup>3+</sup>gcvB<sup>3+</sup>] and ΔgcvAB[pgcvA<sup>3+</sup>gcvB<sup>3+</sup>] strains, with high GcvA and Hfq, the GTs were not significantly different than in the non-transformed strains (Table 2, compare rows 2 and 1 with rows 5 and 6, pH 4.5). The results are consistent with GcvA negatively regulating acid resistance genes and GcvB overcoming the negative effect of GcvA. The results also show GcvA and GcvB affect acid resistance in log phase cells and could play important roles in the ability of enteric organisms to colonize the GI tract.

3.12. Role of GcvB in Cell Physiology. In E. coli, GcvB negatively regulates SstT, CyaA, OppA, and DppA levels, the serine transporter, glycine transporter and the oligopeptide, and dipeptide periplasmic binding proteins, respectively [19, 22, 23]. These proteins not only transport amino acids and peptides to provide nutrients, but possibly toxins and antibiotics [50, 51]. If conditions that favor relatively high levels of amino acids and small peptides also favor the presence of small toxic compounds, the decreased expression...
of transport systems for these small molecules by GcvB could prevent transport of toxic compounds into the cell [22]. Our results show that GcvB also positively regulates genes involved in acid resistance. In addition, GcvA, the activator for gcvB expression, negatively regulates genes involved in acid resistance. These findings suggest GcvB and GcvA play important roles in the ability of E. coli to survive low pH conditions. Recently, in a screen of a sRNA gene knockout library, GcvB was shown to enhance E. coli survival at low pH [25]. Thus, GcvB likely allows E. coli to respond to and survive two stress conditions, the presence of toxic compounds and low pH environments. Both of these conditions are encountered as E. coli moves from an external environment into the GI tract. Understanding the biological roles of GcvB and GcvA in acid resistance and their mechanism(s) of regulation will provide insights as to how cells respond to environmental challenges to infect host organisms.

Acknowledgments

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References


Table 2: Effect of GcvA and GcvB on growth at low pH.

<table>
<thead>
<tr>
<th>Strain</th>
<th>GT (min) grown in LB at pH 7.0</th>
<th>pH 4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) WT</td>
<td>52 ± 5</td>
<td>133 ± 2</td>
</tr>
<tr>
<td>(2) ΔgcvAB</td>
<td>56 ± 2</td>
<td>160 ± 6</td>
</tr>
<tr>
<td>(3) WT[pgcVA^3+]</td>
<td>67 ± 7</td>
<td>306 ± 9</td>
</tr>
<tr>
<td>(4) ΔgcvAB[pgcVA^3+]</td>
<td>60 ± 6</td>
<td>437 ± 45</td>
</tr>
<tr>
<td>(5) WT[pgcVA^3^9 gcVB^3+]</td>
<td>67 ± 7</td>
<td>157 ± 4</td>
</tr>
<tr>
<td>(6) ΔgcvAB[pgcVA^3^9 gcVB^3+]</td>
<td>62 ± 1</td>
<td>150 ± 5</td>
</tr>
</tbody>
</table>

* Cultures were tested at the end of the experiment to verify the pH had not changed.


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