Research Article

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

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In Escherichia 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 Smal site that hybridized to DNA beginning at codon 7 within the hdeA gene. The PCR amplified DNA fragment was digested with EcoRI + Smal and the 303 bp EcoRI-Smal fragment ligated into the EcoRI-Smal sites of plasmid pMC1403 [37], fusing the first 7 codons of the hdeA gene in frame with the 8th codon of the lacZYA genes in pMC1403 (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 pphdeA::lacZ. A 5,574 bp EcoRI-MfeI fragment from pphdeA::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 Smal 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 phdeB::lacZ and phage λhdeB::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 DNA fragment was ligated into the EcoRI and HindIII sites of plasmid pGCVB- lacZ+50 [19], replacing the gcvB fragment with the hdeA fragment, generating plasmid phdeA::lacZ. The cloned sequence was verified by DNA sequence analysis. A 5,538 bp EcoRI-MfeI fragment from pphdeA::lacZ carrying the hdeA::lacZYA fusion was then ligated into the EcoRI site of phage λgt2 [30], generating λhdeA::lacZ. The 3 fusion phages were used to transduce E. coli host strains as described [38]. Each lysogen was tested to ensure it carried a single-copy of the λ chromosome by infection with λcl90c17 [39]. All lysogens were grown at 30°C since all fusion phage carry the λcl857 mutation, resulting in a temperature-sensitive lacI repressor [30]. The λBAD::hdeA::lacZ fusion, where hdeA transcription is under control of the P_BAD promoter, was constructed as described in Figure 1(b).

Plasmid pGS611 (pGCVB+), carrying the E. coli gcvA gene on a 1,155 bp EcoRI fragment, was constructed as follows. In a PCR reaction, an upstream primer was used containing an EcoRI site and that hybridized to a region beginning 121 bp upstream of the gcvA transcription start site and a downstream primer containing an EcoRI site and hybridized to a region beginning 44 bp downstream of the gcvA translation stop codon. The EcoRI sites were added as parts of the primers. The PCR-generated fragment was digested with EcoRI and cloned into the EcoRI site in plasmid pACYC184 [40] and verified by DNA sequence analysis (Figure 1(c)). Plasmid pGS624 (pGCVB+ gcvB+), carrying both the gcvA and gcvB genes, was constructed in the same way except the upstream primer hybridized to DNA 51 bps after the gcvB transcription terminator and the downstream primer hybridized to DNA 44 bps after the gcvA translation stop codon, generating a 1,347 bp EcoRI fragment (Figure 1(c)).

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 (OD\(_{600}\) ~ 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 pGCVB+ (constitutively produces GcvB), pGCVB+ or pGCVB+ gcvB+ 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 hfrQ is linked to the cyaA gene, with predicted phage P1 cotransduction frequencies of ~78% and ~67%, respectively. P1clr phage prepared on GS854 (argA1::Tn10) was used to transduce ΔgcvB::ΩCM\(^{R}\)hdeA::lacZ to TC\(^{R}\) and transductants scored on CM versus TC plates. A TC\(^{R}\) CM\(^{S}\) transductant was purified. P1clr prepared on GS776 (cycA::Tn10) was used to transduce ΔhfrQ::ΩCM\(^{S}\)hdeA::lacZ to TC\(^{R}\) and transductants scored on CM versus TC plates. A TC\(^{R}\) CM\(^{S}\) transductant was purified.
Table 1: Strains, plasmids, and phage.

| Strain, plasmid, or phage | Relevant genotype | Source |
|---------------------------|-------------------|--------|
| Strain*                   |                   |        |
| GS162                     | WT                | This lab |
| GS76                      | cyaA30::Tn10      | This lab |
| GS854                     | argA81::Tn10      |        |
| GS998                     | GS162 gcvA        |        |
| GS1132                    | GS162 Δ(gcvA:gcvB)::aadA (referred to as ΔgcvAB) | [19] |
| GS1144                    | GS162 ΔgcvB::ΩCM8 (referred to as ΔgcvB) | [21] |
| GS1148                    | GS162 hfq-1::ΩCM8 (referred to as Δhfq) | [23] |
| Plasmid                   |                   |        |
| pGS554                    | Single-copy vector + constitutive gcvB (pgcvB2+) | [19] |
| pGS571                    | Multicopy vector + WT gcvB (pgcvB3+) | [29] |
| pGS594                    | Single-copy vector + WT gcvB (pgcvB3+) | This lab |
| pGS609                    | Multi-copy vector + WT hfq (phfq3+) | [23] |
| pGS611                    | Multi-copy vector + WT gcv (pgcvA3+) | This study |
| pGS624                    | Multi-copy vector + WT gcvA gcvB (pgcvA3+gcvB3+) | This study |
| Phage                     |                   |        |
| λgt2                      | λ cloning vector; cL857 repressor | [30] |
| λhdeA::lacZ               | λ vector carrying WT hdeA::lacZ fusion | This study |
| λhdeB::lacZ               | λ vector carrying WT hdeB::lacZ fusion | This study |
| λhdeA36::lacZ             | λ vector carrying hdeA36::lacZ translational 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 f18B5301 ptsF25 rbsR mutations.

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 ΔgcvAB strain were grown for 24 hr at 30°C in LB and tested for acid resistance by dilution into LB at pH 2.0 [43]. The WT was killed significantly more readily at pH 2 than the ΔgcvAB strain (Figure 2, compare black and gray lines). However, when the ΔgcvAB strain was transformed with pgcvB2+ that constitutively expresses GcvB [19], we did not see complementation that restored acid sensitivity (Figure 2, green line). When transformed with the multi-copy plasmid pgcvA3+ gcvB3+, both the WT and the ΔgcvAB transformants were more acid sensitive (Figure 2, compare the black and blue lines and the gray and purple lines). Plasmid pgcvA3+, which carries only the gcvA gene, also complemented the ΔgcvAB mutation, increasing acid sensitivity (Figure 2, compare the gray and red lines). The results suggest it is the absence of GcvA that is responsible for increased acid resistance in the ΔgcvAB strain. It was reported previously that GcvB plays a positive role in 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[pfq3+] 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.
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 pgcvB+ 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 level of expression as the WT lysogen (Figure 3(a), compare lanes 1, 12 and 13). Thus, despite the failure of pgcvB+ 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 GcvA− [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 and the presence of GcvA, suggesting a negative role for GcvA in the hdeA::lacZ fusion.

(c) The gcvA gene is also shown [32]. Two putative GcvA binding sites are indicated in red. The fusion sites for the λhdeA::lacZ transcriptional fusion, the λhdeA::lacZ transcriptional fusion, and the λPBAD::hdeA::lacZ fusion (see below) are indicated with black, red, and green arrows, respectively. (b) Construction of a λPBAD::hdeA::lacZ promoter fusion. The WT λPBAD and λPheA promoters are shown in the top line. The transcription start sites are in red [31, 36]. Small case letters show bases added during PCR amplification of the λPBAD and λPheA promoters. The λPBAD promoter was amplified with an upstream primer containing an EcoRI site at bp +272 relative to the transcription start site (not shown) and a downstream primer with a BsaI site (blue). The λPheA promoter was amplified with an upstream primer containing a BsaI site (blue) and a downstream primer containing a Smal site at codon 10 in the hdeA gene (not shown). The arrows indicate cut sites for BsaI. The amplified products were cut with BsaI, mixed, and ligated, generating a fusion of the λPBAD promoter with the +1G residue of the λPheA promoter. The fragment was then digested with EcoRI + SmaI and ligated into the EcoRI-Smal sites of plasmid pMCI403, and subsequently subcloned into λgt2 as described [19]. (c) The gcvA promoter region of the E. coli chromosome. The regions amplified by PCR and cloned into pACYC184 to generate pGS611 (pgcvA+) and pGS624 (pgcvA+ gcvB+) are indicated with bars. See Section 2.2 for details.
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 ΔgcvAΔhdeA::lacZ lysogen with pgcvB3+. In the ΔgcvAΔhdeA::lacZ lysogen, there would be high GcvA levels and no GcvB, and repression of hdeA::lacZ should be greatest. In the ΔgcvA[pgcvA3+] lysogen there was a 5.8-fold reduction of hdeA::lacZ expression compared to WT and a 2.4-fold reduction compared to the ΔgcvA lysogen (Figure 3(a), compare lanes 1, 7 and 9). The results support a role for GcvA in negatively regulating hdeA::lacZ expression.

3.6. Effect of GcvA, GcvB, and Hfq on hdeA::lacZ Expression in GM + Glycine. In E. coli, GcvB represses dppA::lacZ, oppA::phoA, cyaA::lacZ, and sstT::lacZ fusions when cells are grown in LB, but does not significantly repress these fusions when grown in GM + glycerol [19, 22, 23]. However, gcvB is differentially regulated over a 25-fold range in GM supplemented with inosine versus glycerol [19]. We hypothesize some genes respond to GcvB levels in GM media. Microarray data suggested the hdeB mRNA is 1.6-fold higher in WT than a ΔgcvB strain grown in GM + glycerol [22]. In GM + glycerol, 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 + glycerol as observed in LB. The results suggest GcvB positively regulates hdeA in LB and GM + glycerol. The results are important since they confirm GcvB does regulate in GM + glycerol. 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 + glycerol (compare Figures 4(a) and 4(b) with Figures 4(c) and 4(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 ΔgcvB lysogens in both LB and GM + glycerol (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 GcvB.

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 WTΔhdeA::lacZ, and WTΔhdeB::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 $p_{hfq}^{3+}$ resulted in a 2-fold increase in both $\lambda hdeA::\text{lacZ}$ and $\lambda hdeB::\text{lacZ}$ expression (Figures 4(a) and 4(b), line 3). The presence of $pgcVA^{3+}$ resulted in a 3.5-fold and a 2.2-fold reduction in $\lambda hdeA::\text{lacZ}$ and $\lambda hdeB::\text{lacZ}$ expression, respectively (Figures 4(a) and 4(b), line 4). The presence of plasmid $pgcVA^{3+} \ gcvB^{3+}$ reduced $\lambda hdeA::\text{lacZ}$ and $\lambda hdeB::\text{lacZ}$ expression, but not to the levels of the $pgcVA^{3+}$ 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 $pgcVB^{3+}$ transformant did not show increased expression of $\lambda hdeB::\text{lacZ}$ 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 $hdeAB$ operon and GcvA negatively regulating the operon.

3.9. GcvA, GcvB, and Hfq Regulate $\lambda hdeA::\text{lacZ}$ 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 $\lambda hdeA$ GcvA,
GcvB and Hfq function, we constructed a $\lambda P_{BAD}:hdeA::lacZ$ 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 $\beta$-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 $\lambda hdeA::lacZ$ lysogen grown in LB (Figure 3) and confirmed the fusion is inducible by arabinose. We then lysogenized WT, $\Delta gcvB$, $\Delta gcvAB$, and $\Delta hfq$ 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 $\beta$-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 $\lambda hdeA::lacZ$ mRNA transcript. There was no significant difference in $P_{BAD}:hdeA::lacZ$ expression in the WT, WT[$pgcVA^{+}$] and WT[$pgcVA^{+} gcvB^{+}$] 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 pgcvA3+ or pgcvA3+ΔgcvB3+ 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[pgcvA3+] 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[pgcvA3+] transformant, with high GcvA and no GcvB, there was an additional increase in the GT (Table 2, compare rows 3 and 4, pH 4.5 column). In the WT[pgcvA3+ΔgcvB3+] and ΔgcvAB[pgcvA3+ΔgcvB3+] strains, with high GcvA and GcvB, the GTs were not significantly different than in the non-transformed strains (Table 2, compare rows 1 and 2 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 SerT, 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.

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References

[1] D. S. Merrell and A. Camilli, “Acid tolerance of gastrointestinal pathogens,” Current Opinion in Microbiology, vol. 5, no. 1, pp. 51–55, 2002.
[2] M. P. Castanie-Cornet, T. A. Penfound, D. Smith, J. F. Elliott, and J. W. Foster, “Control of acid resistance in Escherichia coli,” Journal of Bacteriology, vol. 181, no. 11, pp. 3525–3535, 1999.
[3] B. M. Hersh, F. T. Farooq, D. N. Barstad, D. L. Blankenhorn, and J. L. Slonczewski, “A glutamate-dependent acid resistance gene in Escherichia coli,” Journal of Bacteriology, vol. 178, no. 13, pp. 3978–3981, 1996.
[4] J. Lin, In Soo Lee, J. Frey, J. L. Slonczewski, and J. W. Foster, “Comparative analysis of extreme acid survival in Salmonella typhimurium, Shigella flexneri, and Escherichia coli,” Journal of Bacteriology, vol. 177, no. 14, pp. 4097–4104, 1995.
[5] P. L. Moreau, “The lysine decarboxylase CadA protects Escherichia coli starved of phosphate against fermentation acids,” Journal of Bacteriology, vol. 189, no. 6, pp. 2249–2261, 2007.
[6] J. W. Foster, “Escherichia coli acid resistance: tales of an amateur acidophile,” Nature Reviews Microbiology, vol. 2, no. 11, pp. 898–907, 2004.
[7] B. Zhao and W. A. Houry, “Acid stress response in enteropathogenic gammaproteobacteria: an aptitude for survival,” Biochemistry and Cell Biology, vol. 88, no. 2, pp. 301–314, 2010.
[8] J. P. Audia, C. C. Webb, and J. W. Foster, “Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria,” International Journal of Medical Microbiology, vol. 291, no. 2, pp. 97–106, 2001.
[9] S. Gong, H. Richard, and J. W. Foster, “YjeE (AdiC) is the arginine-Arginine antiportor essential for arginine-dependent acid resistance in Escherichia coli,” Journal of Bacteriology, vol. 185, no. 15, pp. 4402–4409, 2003.
[10] R. Iyer, C. Williams, and C. Miller, “Arginine-Arginine antiportor in extreme acid resistance in Escherichia coli,” Journal of Bacteriology, vol. 185, no. 22, pp. 6556–6561, 2003.
[11] K. S. Gajiwala and S. K. Burley, “HDEA, a periplasmic protein that supports acid resistance in pathogenic enteric bacteria,” Journal of Molecular Biology, vol. 295, no. 3, pp. 605–612, 2000.
[12] D. S. Merrell, D. L. Hava, and A. Camilli, “Identification of novel factors involved in colonization and acid tolerance of Vibrio cholerae,” Molecular Microbiology, vol. 43, no. 6, pp. 1471–1491, 2002.
[13] W. Hong, W. Jiao, J. Hu et al., “Periplasmic protein HdeA exhibits chaperone-like activity exclusively within stomach pH range by transforming into disordered conformation,” Journal of Biological Chemistry, vol. 280, no. 29, pp. 27029–27034, 2005.
[14] R. Kern, A. Malki, J. Abballah, J. Tagouri, and G. Richarme, “Escherichia coli HdeB is an acid stress chaperone,” Journal of Bacteriology, vol. 189, no. 2, pp. 603–610, 2007.
[15] T. Schneider, T. M. Barbosa, L. M. McMurry, and S. B. Levy, “The Escherichia coli transcriptional regulator MarA directly represses transcription of purA and hdeA,” Journal of Biological Chemistry, vol. 279, no. 10, pp. 9037–9042, 2004.
[16] M. Shin, M. Song, H. R. Joon et al., “DNA looping-mediated repression by histone-like protein H-NS: specific requirement of Eo70 as a cofactor for looping,” Genes and Development, vol. 19, no. 19, pp. 2388–2398, 2005.
[17] D. L. Tucker, N. Tucker, and T. Conway, “Gene expression profiling of the pH response in Escherichia coli,” Journal of Bacteriology, vol. 184, no. 23, pp. 6551–6558, 2002.
[18] J. Itou, Y. Eguchi, and R. Utsumi, “Molecular mechanism of transcriptional cascade initiated by the EvgS/EvgA system in Escherichia coli K-12,” Bioscience, Biotechnology and Biochemistry, vol. 73, no. 4, pp. 870–878, 2009.
[19] M. L. Urbanowski, L. T. Stauffer, and G. V. Stauffer, “The gcvB gene encodes a small untranslated RNA involved in expression of the dipeptide and oligopeptide transport systems in Escherichia coli,” Molecular Microbiology, vol. 37, no. 4, pp. 856–868, 2000.
[20] C. M. Sharma, F. Darfeuille, T. H. Plantinga, and J. Vogel, “A small RNA regulates multiple ABC transporter mRNAs by targeting C/A-rich elements inside and upstream of ribosome-binding sites,” Genes and Development, vol. 21, no. 21, pp. 2804–2817, 2007.
[21] S. C. Pulvermacher, L. T. Stauffer, and G. V. Stauffer, “The role of the small regulatory RNA GcvB in GcvB/mRNA
posttranscriptional regulation of oppA and dppA in Escherichia coli," *FEMS Microbiology Letters*, vol. 281, no. 1, pp. 42–50, 2008.

[22] S. C. Pulvermacher, L. T. Stauffer, and G. V. Stauffer, “Role of the sRNA GcvB in regulation of cyclA in *Escherichia coli*,” *Microbiology*, vol. 155, no. 1, pp. 106–114, 2009.

[23] S. C. Pulvermacher, L. T. Stauffer, and G. V. Stauffer, “The small RNA GcvB regulates ssrT mRNA expression in *Escherichia coli*,” *Journal of Bacteriology*, vol. 91, no. 1, pp. 238–248, 2009.

[24] C. M. Sharma, K. Papenfort, S. R. Perntitzsch, H. J. Mollenkopf, J. C. D. Hinton, and J. Vogel, “Pervasive post-transcriptional control of genes involved in amino acid metabolism by the Hfq-dependent GcvB small RNA,” *Molecular Microbiology*, vol. 81, no. 5, pp. 1144–1165, 2011.

[25] Y. Jin, R. M. Watt, A. Danchin, and J. D. Huang, “Small noncoding RNA GcvB is a novel regulator of acid resistance in *Escherichia coli*,” *BMC Genomics*, vol. 10, article 165, 2009.

[26] S. C. Pulvermacher, L. T. Stauffer, and G. V. Stauffer, “Role of the *Escherichia coli* Hfq protein in GcvB regulation of oppA and dppA mRNAs,” *Microbiology*, vol. 155, no. 1, pp. 115–123, 2009.

[27] R. L. Wilson, P. S. Steiert, and G. V. Stauffer, “Positive regulation of the *Escherichia coli* glycine cleavage enzyme system,” *Journal of Bacteriology*, vol. 175, no. 3, pp. 902–904, 1993.

[28] R. L. Wilson and G. V. Stauffer, “DNA sequence and characterization of GcvA, a LysR family regulatory protein for the *Escherichia coli* glycine cleavage enzyme system,” *Journal of Bacteriology*, vol. 176, no. 10, pp. 2862–2868, 1994.

[29] A. D. Jourdand and G. V. Stauffer, “Genetic analysis of the GcvA binding site in the gcvA control region,” *Microbiology*, vol. 145, no. 8, pp. 2153–2162, 1999.

[30] S. M. Panasenko, J. R. Cameron, R. W. Davis, and I. R. Lehman, “Five hundredfold overproduction of DNA ligase after induction of a hybrid lambda lysogen constructed in vitro,” *Science*, vol. 196, no. 4286, pp. 188–189, 1977.

[31] A. Arnqvist, A. Olsen, and S. Normark, “σ5-dependent growth-phase induction of the cssA promoter in *Escherichia coli* can be achieved in vivo by σ55 in the absence of the nucleoid-associated protein H-NS,” *Molecular Microbiology*, vol. 13, no. 6, pp. 1021–1032, 1994.

[32] F. Hommais, E. Krin, J. Y. Coppee et al., “GadE (YhiE): a novel activator involved in the response to acid environment in *Escherichia coli*;” *Microbiology*, vol. 150, no. 1, pp. 61–72, 2004.

[33] A. Tramonti, M. De Canio, and D. De Biase, “GadX/GadW-dependent regulation of the *Escherichia coli* acid fitness island: transcriptional control at the gadY-gadW divergent promoters and identification of four novel 42 bp GadX/GadW-specific binding sites,” *Molecular Microbiology*, vol. 70, no. 4, pp. 965–982, 2008.

[34] C. Ruiz, L. M. McMurry, and S. B. Levy, “Role of the multidrug resistance regulator MarA in global regulation of the hdeAB acid resistance operon in *Escherichia coli*,” *Journal of Bacteriology*, vol. 190, no. 4, pp. 1290–1297, 2008.

[35] R. L. Wilson, M. L. Urbanowski, and G. V. Stauffer, “DNA binding sites of the LysR-type regulator GcvA in the gcv and gcvA control regions of *Escherichia coli*,” *Journal of Bacteriology*, vol. 177, no. 17, pp. 4940–4946, 1995.

[36] L. M. Guzman, D. Belin, M. J. Carson, and J. Beckwith, “Tight regulation, modulation, and high-level expression by vectors containing the arabinoose P(BAD) promoter,” *Journal of Bacteriology*, vol. 177, no. 14, pp. 4121–4130, 1995.

[37] M. J. Casadan, J. Chou, and S. N. Cohen, “In vitro gene fusions that join an enzymatically active β-galactosidase segment to amino-terminal fragments of exogenous proteins: *Escherichia coli* plasmid vectors for the detection and cloning of translational initiation signals,” *Journal of Bacteriology*, vol. 143, no. 2, pp. 971–980, 1980.

[38] M. L. Urbanowski and G. V. Stauffer, “Autoregulation by tandem promoters of the *Salmonella typhimurium* LT2 metl gene,” *Journal of Bacteriology*, vol. 165, no. 3, pp. 740–745, 1986.

[39] K. Shimada, R. A. Weisberg, and M. E. Gottesman, “Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens,” *Journal of Molecular Biology*, vol. 63, no. 3, pp. 483–503, 1972.

[40] A. C. Y. Chang and S. N. Cohen, “Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid,” *Journal of Bacteriology*, vol. 134, no. 3, pp. 1141–1156, 1978.

[41] J. Miller, *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 1972.

[42] H. J. Vogel and D. M. Bonner, “Acylornithinase of *Escherichia coli*: partial purification and some properties,” *The Journal of Biological Chemistry*, vol. 218, no. 1, pp. 97–106, 1956.

[43] J. Gorden and P. L. C. Small, “Acid resistance in enteric bacteria,” *Infection and Immunity*, vol. 61, no. 1, pp. 364–367, 1993.

[44] M. P. Castanie-Cornet and J. W. Foster, “Escherichia coli acid resistance: CAMP receptor protein and a 20 bp cis-acting sequence control pH and stationary phase expression of the gadA and gadBC glutamate decarboxylase genes,” *Microbiology*, vol. 147, no. 3, pp. 709–715, 2001.

[45] M. P. Castaniti-Cornet, H. Treffandier, A. Franquez-Charlot, C. Gutierrez, and K. Cam, “The glutamate-dependent acid resistance system in *Escherichia coli*: essential and dual role of the His-Asp phosphorelay RcsCDB/AF,” *Microbiology*, vol. 153, no. 1, pp. 238–246, 2007.

[46] J. A. Opdyke, J. G. Kang, and G. Storz, “GadY, a small-RNA regulator of acid response genes in *Escherichia coli*,” *Journal of Bacteriology*, vol. 186, no. 20, pp. 6698–6705, 2004.

[47] R. A. Lease, D. Smith, K. McDonough, and M. Belfort, “The small noncoding DsrA RNA is an acid resistance regulator in *Escherichia coli*,” *Journal of Bacteriology*, vol. 186, no. 18, pp. 6179–6185, 2004.

[48] L. D. Wonderling, M. L. Urbanowski, and G. V. Stauffer, “GcvA binding site 1 in the gcvTHP promoter of *Escherichia coli* is required for GcvA-mediated repression but not for GcvA-mediated activation,” *Microbiology*, vol. 146, no. 11, pp. 2909–2918, 2000.

[49] A. Zhang, K. M. Wassarman, C. Rosenow, B. C. Tjaden, G. Storz, and S. Gottesman, “Global analysis of small RNA and mRNA targets of Hfq,” *Molecular Microbiology*, vol. 50, no. 4, pp. 1111–1124, 2003.

[50] I. D. Hiles, M. P. Gallagher, D. J. Jamieson, and C. F. Higgins, “Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*,” *Journal of Molecular Biology*, vol. 195, no. 1, pp. 125–142, 1987.

[51] M. W. Smith and J. W. Payne, “Simultaneous exploitation of different peptide permeases by combinations of synthetic peptide smugglins can lead to enhanced antibacterial activity,” *FEMS Microbiology Letters*, vol. 58, no. 3, pp. 311–316, 1990.