Research Article

Multiple Roles for the sRNA GcvB in the Regulation of Slp Levels in Escherichia coli

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Received 4 March 2013; Accepted 31 March 2013

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The E. coli gcvB gene encodes a small RNA that regulates many genes involved in the transport of dipeptides, oligopeptides, and amino acids (oppA, dppA, cycA, and sstT). A microarray analysis of RNA isolated from an E. coli wild-type and a ΔgcvB strain grown to midlog phase in Luria-Bertani broth indicated that genes not involved in transport are also regulated by GcvB. One gene identified was slp that encodes an outer membrane lipoprotein of unknown function induced when cells enter stationary phase.

The aim of this study was to verify that slp is a new target for GcvB-mediated regulation. In this study we used RT-PCR to show that GcvB regulates slp mRNA levels. GcvB negatively controls slp: lacZ in cells grown in Luria-Bertani broth by preventing an Hfq-mediated activation mechanism for slp: lacZ expression. In contrast, in glucose minimal medium supplemented with glycine, GcvB is required for inhibition of slp: lacZ expression, and Hfq prevents GcvB-mediated repression. Thus, GcvB regulates slp in both LB and in glucose minimal + glycine media and likely by mechanisms different than how it regulates sstT, dppA, cycA, and oppA. Repression of slp by GcvB results in an increase in resistance to chloramphenicol, and overexpression of slp in a ΔgcvB strain results in an increase in sensitivity to chloramphenicol.

1. Introduction

The E. coli chromosome encodes ~100 small non-translated regulatory RNAs (sRNAs) [1]. A number of these sRNAs have been shown to function as regulators of outer membrane proteins and therefore play important roles in stress responses and virulence gene regulation [2–4]. In addition, most of these sRNAs regulate expression of target genes posttranscriptionally by base pairing with the target mRNAs [5]. The Hfq protein is required for regulation by this class of sRNAs [6]. In most cases, base pairing results in negative regulation of translational activity and altered stability of the target mRNA [5]. However, DsrA and RprA bind to rpoS mRNA, likely preventing formation of an inhibitory secondary structure that sequesters the ribosome-binding site, resulting in increased translation [7–9].

The gcvB gene encodes a nontranslated RNA of 206 nucleotides (nts) [10]. Transcription of the gcvB gene is activated by the GcvA protein when the cellular level of glycine is high and repressed by GcvA when the cellular level of glycine is limiting; repression requires an additional protein GcvR [10]. The production of GcvB is highest during the log phase of growth, and GcvB is not detectable in stationary phase cells [12]. In E. coli, GcvB regulates genes involved in the transport of amino acids and small peptides [10, 11, 14, 15]. GcvB also plays a role in acid resistance, although the precise mechanism is unknown [16, 17]. Recently it was shown that GcvB acts as a negative regulator of CsgD, the master regulator of curli and cellulose synthesis [18]. In Salmonella enterica serovar Typhimurium, GcvB has been validated to regulate more than 20 targets and possibly acts as a regulatory node in amino acid metabolism [19, 20]. GcvB represses many of its target mRNAs at the posttranscriptional level by an antisense mechanism, and repression requires the Hfq protein [14, 15, 20–22]. The sRNA RyhB regulates as many as 18 operons encoding 56 genes [23], and many other sRNAs are also predicted to regulate more than one target [24]. Thus, it is likely that GcvB regulates other genes in E. coli. To identify additional regulatory targets for E. coli GcvB, we compared RNA isolated from a wild type (WT) and an otherwise
Table 1: Strains and plasmids.

| Strain or plasmid | Relevant genotype | Source or reference |
|-------------------|-------------------|---------------------|
| Strain*           |                   |                     |
| GS162             | WT                | This laboratory      |
| GS1132            | GS162 ΔgcvAΔgcvB  | [10]                |
| GS1144            | GS162 ΔgcvB::ΔCM  | [11]                |
| GS1148            | GS1142 ΔgcvB::ΔCM | [12]                |
| GS1149            | GS1142 ΔgcvB::ΔCMt| [12]                |
| Plasmid           |                   |                     |
| pGS554            | Single-copy vector + WT gcvB with a constitutive promoter (pgcvB<sup>2+</sup>) | [10] |
| pGS571            | Multicopy vector + WT gcvB (pgcvB<sup>2+</sup>) | [13] |
| pGS594            | Single-copy vector + WT gcvB (p<sup>gcvB</sup><sup>+</sup>) | This laboratory |
| pGS609            | Multicopy vector + WT hfq (p<sup>hfq</sup><sup>2+</sup>) | [14] |
| pGS631            | pGS594 with a change in gcvB at bp +131 and +132 of TT to CC (p<sup>gcvB</sup> + 131CC) | [15] |
| pGS632            | pGS594 with a change in gcvB at bp +142 and +143 of TG to CA (p<sup>gcvB</sup> + 142CA) | [15] |
| pGS633            | pGS594 with a change in gcvB at bp +159 and +160 of TG to CC (p<sup>gcvB</sup> + 159CC) | [15] |
| pGS634            | pGS594 with a change in gcvB at bp +142 and +143 of TG to CA and bp +159 and +160 of TG to CC (p<sup>gcvB</sup> + 142CA + +159CC) | [15] |

*All strains also carry the pheA905 thi araD129 rpsL150 relA1deoC1flbB5301 ptsF25 rbsR mutations.

2. Materials and Methods

2.1. Bacterial Strains, Plasmids, and Phage. The *E. coli* strains and plasmids used in this study are listed in Table 1 or are described in the text. The λ<sub>slp</sub>:lacZ translational fusion was constructed by PCR as follows. A DNA fragment was synthesized using primer Slp-EcoRI-5'GGGGTGGAAATA-GAAAATACCATC that hybridized to *E. coli* DNA upstream of an EcoRI site that begins 288 base pairs (bps) upstream of the *slp* translation start site and primer S-Smal 5'-CCTAAAACGGGGGAAGATGTCACCTTGTGC with a Smal site (underlined) and that hybridized to DNA beginning at codon 10 within the *slp* structural gene (Figure 1A). The amplified fragment was digested with EcoRI + Smal and the 320 bp EcoRI-Smal fragment purified from a 1% agarose gel and ligated into the EcoRI-Smal sites of plasmid pMC1403 [29], fusing the first 10 codons of the *slp* structural gene in frame with the 8th codon of the lacZ gene in pMC1403. The fusion was verified by DNA sequence analysis at the DNA Core Facility of the University of Iowa. The intermediate plasmid was designated pSlp::lacZ. A 5,771 bp EcoRI-MfeI fragment from pSlp::lacZ carrying the *slp*:lacZ fusion and lacYA genes was then ligated into the EcoRI site of phage λgt2 [30]. The phage was used to lysogenize appropriate *E. coli* host strains as described previously [31]. Each lysogen was tested to ensure that it carried a single copy of the λ chromosome by infection with λcI90c17 [32]. All lysogens were grown at 30°C, since all fusion phage carry the λcI857 mutation, resulting in a temperature sensitive λcI repressor [30].

2.2. Media. The rich medium used was Luria-Bertani broth (LB) [33]. The defined medium used was Vogel and Bonner minimal salts [34] supplemented with 0.4% glucose (GM). GM medium was always supplemented with phenylalanine, glycine, and thiamine, since all strains carry the pheA905 and thi mutations and glycine induces gcvB expression [10]. Agar was added at 1.5% for solid media. Supplements were added at the following concentrations (μg mL<sup>-1</sup>): phenylalanine, 50; glycine, 300; thiamine, 1; chloramphenicol (CM), 3; ampicillin (AP), 50 for single-copy plasmids and 150 for multicopy plasmids.

2.3. Enzyme Assays. β-galactosidase assays were performed on cultures harvested at midlog phase of growth (OD<sub>600</sub> ~ 0.5) using the chloroform/SDS lysis procedure [33]. Results are the averages of two or more assays with each sample done in triplicate.

2.4. CM Sensitivity Assay. CM sensitivity was tested by the agar disk diffusion method. Cells were grown overnight, and ~5 x 10<sup>8</sup> cells spread on Mueller-Hinton plates. CM disks
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TAGTAAC ATG AAC ATG ACA AAA GGT GCA CTC ATC CTC AGC CTT TCA TTT TTG CTT

Start

Translational fusion site

Figure 1: The slp control region sequence. (a) slp. The promoter –35 and –10 sequences, the +1 transcription initiation site, Shine-Dalgarno (SD) sequence, and translation start codon are underlined [25]. The translation fusion point at codon 10 of slp with codon 8 of lacZ is indicated with an arrow. The upstream fusion site occurs 288 bps upstream of the transcription start site (not shown). (b) Comparison of GcvB between slp mRNA. The AUG translation initiation site and SD sequence for slp are indicated above the sequence. Complementarity between GcvB and slp mRNA is indicated with lines between the sequences and GU bps with dots. Separate changes made to gcvB [15] are indicated below the sequence and are color coded.

3. Results

3.1. The slp Gene Is a Target for GcvB and Hfq Regulations.

Microarray analysis of RNA isolated from an E. coli WT strain and an otherwise isogenic ΔgcvB strain grown in LB suggested that Slp, an outer membrane lipoprotein of unknown function [25], is negatively regulated by GcvB [15]. To confirm the microarray analysis that slp is regulated by GcvB, we carried out RT-PCR. WT strain GS162, the ΔgcvB strain GS1144, and GS1144 transformed with the single-copy plasmid pGS594 carrying WT gcvB (pgcvB +) were grown in LB at 30°C to midlog phase of growth (OD600 ~ 0.5) and total cellular RNA isolated. Samples of 0.5 μg of RNA from each culture were used in an RT-PCR assay. We observed an increased level of DNA corresponding to the slp mRNA amplified from the ΔgcvB sample compared to the level of DNA amplified from either the WT or ΔgcvB complemented strain (Figure 2, compare lane 3 with lanes 2 and 4). The results agree with the microarray analysis indicating that GcvB negatively regulates slp mRNA levels in cells grown in LB. As a control, we added primers to the RT-PCR reactions to amplify ompT mRNA; ompT was not identified by microarray analysis to be regulated by GcvB. We observed roughly the same levels of amplified DNA corresponding to the ompT mRNA from each strain (Figure 2). These data confirm that GcvB does not regulate ompT mRNA levels in LB and that the increased slp mRNA levels observed in the ΔgcvB strain are due to the absence of GcvB.

3.2. Regulation of slp::lacZ in LB.

To confirm that GcvB negatively regulates slp, we constructed a slp::lacZ translational fusion (Figure 1(a)). The fusion was cloned onto phage λgt2, and the fusion phage used to lysogenize the WT strain GS162 and the ΔgcvB mutant strain GS1144. The lysogens were grown in LB to midlog phase of growth (OD600 ~ 0.5), and the cultures assayed for β-galactosidase activity. Expression
of the slp::lacZ fusion in the WT lysogen was about 2-fold lower than in the ΔgcvB lysogen (Figure 3(a), lanes 1 and 2). Transformation of the WT lysogen with plasmid pGS594, a single-copy plasmid carrying WT gcvB, reproducibly reduced slp::lacZ expression compared to the nontransformed WT strain (Figure 3(a), compare lanes 1 and 5). Transformation of the ΔgcvB lysogen with pGS594, however, only partially restored the level of expression to that of the WT lysogen (Figure 3(a), compare lanes 1, 2 and 6). It is unknown why the single-copy plasmid failed to fully complement the ΔgcvB mutation. These results, however, support the microarray assay and RT-PCR data that GcvB has a negative role in slp expression.

The Hfq protein is required for regulation by sRNAs that base pair with target mRNAs [6]. Thus, the slp::lacZ fusion was also used to lysogenize the Δhfq mutant strain GS1148. The lysogen was grown in LB to midlog phase of growth and assayed for β-galactosidase activity. The expression of the slp::lacZ fusion in the Δhfq lysogen was reduced compared to the level in the WT lysogen (Figure 3(a), compare lanes 1 and 3). Transformation of the WT lysogen and the Δhfq lysogen with multicyclic plasmid phfq3+(pGS609) resulted in about a 2-fold increase in slp::lacZ expression compared to the WT lysogen (Figure 3(a), compare lane 1 with lanes 7 and 8). These results suggest that Hfq has a positive role in slp expression.

Two models could explain the pervious results for a positive role of Hfq in slp::lacZ expression. GcvB RNA could directly reduce slp::lacZ expression, and the role of Hfq could be to antagonize this repression. Alternatively, Hfq could act to directly activate slp::lacZ expression, and GcvB could function in some manner to block Hfq activation. To test which model, if either, is correct, we lysogenized the ΔgcvBΔhfq double mutant GS1149 with λslp::lacZ, grew the lysogen in LB to midlog phase of growth, and assayed for β-galactosidase activity. We hypothesized that if the role of Hfq is merely to prevent GcvB repression, then slp::lacZ expression should be high in the double mutant without GcvB, whereas if GcvB functions to block Hfq activation, then slp::lacZ expression should be low in the double mutant without Hfq. Expression of slp::lacZ was reduced in the double mutant compared to WT (Figure 3(a), compare lanes 1 and 4). The results suggest that Hfq is required to elevate slp::lacZ expression.

Two additional experiments support a positive role for Hfq in slp::lacZ expression. If GcvB directly represses slp::lacZ rather than antagonize Hfq activation, we hypothesized that high levels of GcvB would superrepress slp::lacZ in the absence of Hfq. We transformed the Δhfq lysogen with plasmids pgcvB+(pGS594), pgcvB+ (pGS554), and pgcvB3+ (pGS571). pgcvB+ is a single-copy plasmid that expresses gcvB from the native gcvB promoter, pgcvB+ is a single-copy plasmid that constitutively expresses gcvB [10], and pgcvB3+ is a multicopy plasmid that overexpresses gcvB [13]. Expression of slp::lacZ in the three transformants was only slightly lower than in the WT lysogen (Figure 3(a), compare lane 1 with lanes 9, 10, and 11). The results suggest that GcvBs role is not to directly repress slp::lacZ.

We then transformed the ΔgcvB and ΔgcvBΔhfq lysogens with multi-copy plasmid phfq3+(pGS609) carrying WT hfq. If Hfq is a positive regulator of slp::lacZ, and GcvB functions to block Hfq activation, then slp::lacZ should be overexpressed in the presence of high levels of Hfq and in the absence of GcvB. Expression of slp::lacZ was more than 4-fold higher in the transformants than in the WT lysogen (Figure 3(a), compare lane 1 with lanes 12 and 13). The results are in agreement with Hfq positively regulating slp::lacZ, and the role of GcvB is to prevent activation by Hfq. Whether Hfq directly interacts with slp mRNA to increase stability or to increase translation is unknown, Hfq has been shown to be able to act alone as a translational repressor of mRNA [36, 37]. Thus, it is possible that Hfq alone could bind slp mRNA to increase expression. We have purified Hfq and are in the process of making slp mRNA and GcvB RNA to run in vitro gel mobility shift experiments to test whether Hfq binds slp mRNA and determine if GcvB competes with this binding. Also, Hfq could interact with an additional sRNA to regulate slp mRNA levels. We used the TargetRNA program [38] to search the E. coli chromosome for a possible sRNA that could base pair with the slp mRNA, but found no significant matches. However, if small or noncontiguous sequences are involved in the sRNA/slp mRNA interaction, the identification of this putative sRNA could have been missed in the search.

Many E. coli sRNAs that use base pair to regulate gene expression require Hfq [6]. It was shown previously that GcvB repression of oppA, dppA, cycA, and ssTm mRNAs is Hfq-dependent [11, 14, 15], and it is known that GcvB interacts with Hfq [39]. The same results were reported for S. typhimurium [19]. If the role of GcvB in slp regulation is to bind Hfq and prevent Hfq activation of slp, we predicted that in a ΔgcvBΔhfq mutant, there would not be a significant increase in slp mRNA levels. We carried out RT-PCR on total RNA isolated from the ΔgcvBΔhfq strain GS1149 grown in LB at 30°C to midlog phase of growth (OD600 ~ 0.5). As expected, we did not observe an increased level of DNA corresponding
to slp mRNA amplified from the ΔgcvBΔhfq sample and possibly a decreased level, compared to the level of DNA amplified from the WT strain (Figure 2, compare lanes 2 and 5). We will use qRT-PCR to test if there is less slp mRNA in the ΔgcvBΔhfq strain compared to the WT. The results, however, are in agreement with the β-galactosidase assays (Figure 3(a)) suggesting that Hfq is required for increased slp expression in LB.

3.3. Regulation of slp: lacZ in GM + Glycine Medium. Significant repression of dppA: lacZ, oppA: phoA, and cyaA: lacZ fusions by GcvB was only observed in WT lysogens grown in LB, with little or no repression seen when the lysogens were grown in GM + glycine [10, 15]. Repression of an sstT::lacZ fusion by GcvB in a WT lysogen grown in LB was ~12-fold, and repression of the WT lysogen in GM + glycine was ~2-fold [14]. Because the gcvB gene is regulated over a 25-fold range in GM media [10], we tested if GcvB and Hfq are able to regulate slp: lacZ in GM medium. The lysogens used previously were grown in GM + glycine to midlog phase of growth (OD_{600} ~ 0.5) and assayed for β-galactosidase activity. Expression of slp: lacZ in WT was ~7-fold higher in GM + glycine compared to LB grown cells (Figures 3(a) and 3(b)). In addition, β-galactosidase levels in the WT lysogen were about 2-fold lower compared to the ΔgcvB lysogen (Figure 3(b), lanes 1 and 2). Transformation of the WT lysogen with the single-copy plasmid pgcvb′ consistently reduced slp: lacZ expression compared to the nontransformed WT lysogen (Figure 3(b), compare lanes 1 and 5). Transformation of the ΔgcvB lysogen with pgcvb′ reduced slp: lacZ expression, although full complementation was not observed (Figure 3(b), compare lanes 2 and 6). The results suggest that GcvB has a negative role in slp: lacZ expression in cells grown in GM + glycine as well as in LB.

Expression of slp: lacZ in the Δhfq lysogen was consistently lower than in the WT lysogen (Figure 3(b), lanes 1 and 3). Transformation of the Δhfq lysogen with multicopy pphf3 restored slp: lacZ expression to slightly higher levels than in the WT lysogen (Figure 3(b), compare lanes 1 and 7). The results suggest Hfq is required for normal slp: lacZ expression in GM + glycine. However, expression of slp: lacZ in the ΔgcvBΔhfq double mutant was 2-fold higher than in the WT lysogen (Figure 3(b), compare lanes 1 and 4). This is opposite of the result observed in LB (Figure 3(a), lanes 1 and 4) and suggests that GcvB and Hfq have different roles in GM + glycine compared to LB.

One model to explain the previous results is that GcvB negatively regulates slp: lacZ in GM + glycine, and the role of Hfq is to block GcvB repression rather than to activate slp: lacZ expression as in LB. To test this hypothesis, the Δhfq lysogen was transformed with plasmids pgcvb′ (pGS594), pgcvb′2 (pGS554), and pgcvb′2+(pGS571), the transformants grown in GM + glycine to midlog phase of growth (OD_{600} ~ 0.5) and assayed for β-galactosidase activity. We hypothesized that high levels of GcvB would superrepress slp: lacZ in the absence of Hfq. Expression of slp: lacZ in the transformants was reduced significantly compared to the WT lysogen (Figure 3(b), compare lane 1 with lanes 8–10). The results suggest that GcvB does not negatively regulate slp: lacZ levels in GM + glycine, and the role of Hfq is likely to prevent GcvB repression.

3.4. The slp Leader Sequence. Most sRNAs affect translation of target mRNA molecules, although the precise
Table 2: GcvB alters sensitivity to CM.*

| Relevant genotype        | Disk potency (30 μg CM) Zone diameter (mm) | P value compared to the WT strain | GT (min) grown in: |
|--------------------------|-------------------------------------------|----------------------------------|-------------------|
| WT                       | 22.19 ± 0.92                             |                                  | LB                |
| ΔgcvAΔgcvB               | 23.31 ± 0.88                             | 0.0107                           | 198 ± 23          |
| WT[pgcvB3+]              | 21.06 ± 0.56                             | 0.0260                           | 170 ± 5           |

* WT (GS62), ΔgcvAΔgcvB (GS132), and WT transformed with pgcvB3+ were grown overnight at 30°C (AP was added for the plasmid-carrying strain) and tested for CM sensitivity on Mueller-Hinton plates (see Materials and Methods). In addition, 30 μL of each overnight culture was used to inoculate 5 mL of LB with or without 3 μg mL⁻¹ CM, the cultures grown at 30°C and generation times (GT) measured. Average inhibition zones and standard deviations of eight Mueller-Hinton plates and average generation times and standard deviations of 3 independent growth experiments are reported.

mechanism(s) remain(s) unknown. Thus, it makes sense that sRNAs with either known or predicted targets base pair to the 5’-untranslated region near or overlapping the translation initiation site (e.g., RyhB/sodB mRNA, DsrA/rpoS mRNA) [8, 24]. In addition, these target mRNAs usually have long 5’-untranslated regions that normally can fold into distinct secondary structures. The slp mRNA has a short untranslated region of 25 nts [25]. This sequence, including the first 20 nts of the coding region, does not fold into any distinct secondary structure by the mfold program [40, 41]. A comparison of the slp mRNA to GcvB RNA did not identify any region of significant complementarity between the two RNAs. However, small noncontiguous regions of complementarity between the two RNAs around the slp translation start codon and nts +125 to +177 of GcvB were identified (Figure 1(b)). Thus it is possible that GcvB/mRNA pairing is part of the mechanism employed by GcvB for negative regulation of slp mRNA in GM + glycine. Previously, we mutated nucleotides in GcvB predicted to disrupt base pairing with cya mRNA [15]. Since these mutations also disrupt putative base pairing of GcvB with slp mRNA, we determined if there was an effect of the mutations on slp::lacZ expression. We transformed the ΔgcvB λslp::lacZ lysogen with single-copy plasmids carrying WT gcvB or various mutations in gcvB between nts +125 and +177 that disrupt complementarity between GcvB and slp mRNA (Figure 1(b)). The strains were grown in GM + glycine (+ AP for the complemented strains) to midlog phase of growth (OD₆₀₀ ~ 0.5) and assayed for β-galactosidase activity. However, none of the gcvB mutations predicted to alter base pairing between GcvB and slp::lacZ mRNA altered expression of slp::lacZ compared to WT GcvB (data not shown). Recently it was shown that a high degree of redundancy exists in the E. coli and S. enterica GcvBs [20, 22]. Thus, it is possible that more extensive changes are required to prevent GcvB repression or a few critical base pairing interactions necessary for repression were not changed in the mutational analysis. It is also possible that GcvB does not regulate slp::lacZ directly in GM + glycine media but regulates an additional factor necessary for repression.

3.6. Effects of GcvB Levels on CM Resistance. In addition to expression of slp being downregulated by the Mar system [27, 28], a null slp mutant showed a modest increase in CM resistance and a strain that overproduced Slp was more sensitive to CM [26]. We hypothesized that a ΔgcvB strain that overproduces Slp would be more sensitive to CM than a WT strain, and a strain carrying multicopy plasmid pgcvB3+ that overproduces GcvB RNA would produce less Slp and be more resistant to CM. We tested the effect of CM on growth of WT (GS62), ΔgcvAΔgcvB (GS132), and WT transformed with plasmid pgcvB3+ (pGS571). We used the ΔgcvAΔgcvB strain since it carries a spectinomycin resistance cassette, whereas the ΔgcvB strain (GS1144) carries a CM resistance cassette. We showed previously that dppA::lacZ and oppA::phoA fusions are regulated similarly in a ΔgcvB strain and a ΔgcvAΔgcvB strain [12]. In an agar diffusion disk assay, the ΔgcvAΔgcvB strain showed a modest but statistically significant increase in CM sensitivity, and the WT (pgcvB3+) strain showed a modest but statistically significant decrease in CM sensitivity relative to the WT strain (Table 2). Because the changes were small, we also tested the previous strains in a growth experiment. The generation times (GTs) of the 3 strains in
LB were essentially the same (Table 2). Thus, neither the ΔgcvAΔgcvB allele nor plasmid p𝑔𝑐V𝐵3+ had any significant effect on growth rate. The presence of 3 µg mL-1 CM added to LB significantly increased the GTs of all 3 strains. In addition, we saw a consistent increase in the GT of the ΔgcvAΔgcvB strain relative to WT and a consistent decrease in the GT of WT (pgcvB3+) compared to the WT strain (Table 2). Although the differences in generation times are modest, in conjunction with the results from the diffusion disk assay, suggest that negative regulation of Slp levels by GcvB alters the sensitivity to CM.

4. Discussion

In both E. coli and S. enterica, genes shown to be regulated directly by GcvB are controlled by an antisense mechanism, and many are involved in the transport of amino acids and small peptides [10, 11, 14, 19, 20]. Since GcvB is only expressed in log-phase cells when nutrients are plentiful [12, 19], it likely controls the uptake of amino acids, peptides, and small toxic molecules under this condition to optimize cell growth. The slp gene encodes an outer membrane protein of unknown function [25]. Thus, Slp possibly does not fit the typical class of target genes controlled by GcvB. The localization of Slp in the outer membrane suggests a potential role in protecting stationary phase cells from environmental stress or facilitating nutrient availability in the periplasm. Since GcvB is not detected in stationary phase cells [12, 19], our results suggest that the role of GcvB is to keep Slp levels low in exponentially growing cells. The disappearance of GcvB in stationary phase would allow a more rapid induction of slp expression.

In LB grown cells, the mechanism of regulation of slp::lacZ by GcvB appears to be by sequestration of Hfq, which is required for full expression of slp::lacZ, rather than by an antisense mechanism (Figure 3(a)). In GM + glycine, however, GcvB negatively regulates slp::lacZ, and the role of Hfq appears to be to block repression by GcvB (Figure 3(b)). The ability of GcvB to repress slp::lacZ expression in the absence of Hfq suggests that GcvB might function alone or with a different chaperone to regulate this system. In Staphylococcus aureus, RNAIII functions to repress the synthesis of virulence factors without a requirement for Hfq, suggesting another protein chaperone [47, 48]. If GcvB regulates slp expression in GM + glycine without Hfq, it possibly does so in a way that is mechanistically different from the way it regulates other known target mRNAs [11, 12, 14, 15, 18, 20]. Verification of this model for regulation of slp would demonstrate that GcvB is a more versatile regulatory RNA than previously thought.

Acknowledgments

This work was supported by the Vice President for Research of the Carver College of Medicine and the Department of Microbiology of the University of Iowa.

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