GcvR interacts with GcvA to inhibit activation of the *Escherichia coli* glycine cleavage operon

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The *Escherichia coli* glycine cleavage enzyme system, encoded by the *gcvTHP* operon, catalyses the oxidative cleavage of glycine to CO₂, NH₃ and a one-carbon methylene group. Transcription of the *gcv* operon is positively regulated by GcvA and negatively regulated by GcvA and GcvR. Using a LexA-based system for analysing protein heterodimerization, it is shown that GcvR interacts directly with GcvA *in vivo* to repress *gcvTHP* expression. Several mutations in either *gcvA* or *gcvR* that result in a loss of *gcv* repression also result in a loss of GcvA/GcvR heterodimerization. Finally, it is shown that the C-terminal half of GcvA is involved in its interaction with GcvR, whilst the entire GcvR protein appears to be necessary for heterodimerization.

Keywords: *gcvTHP*, repressor, activator, repression, antiactivation

INTRODUCTION

The *Escherichia coli* glycine cleavage enzyme system, encoded by the *gcvTHP* (*gcv*) operon, catalyses the oxidative cleavage of glycine to CO₂, NH₃ and a one-carbon methylene group (Kikuchi, 1973; Meedel & Pizer, 1974). This one-carbon group is transferred to the acceptor tetrahydrofolate to form 5,10-methylenetetrahydrofolate, a methyl donor used in the biosynthesis of purines, thymine, methionine and numerous methylated products (Mudd & Cantoni, 1964). Transcription of the *gcv* operon is activated by the GcvA protein in the presence of excess glycine. When glycine is limiting, GcvA actively represses expression of the operon; this repression is more severe in the presence of excess purines (Wilson *et al.*, 1993a, b). GcvA, a member of the LysR family of transcriptional regulators, binds by way of a helix–turn–helix motif to three sites upstream of the *gcv* promoter (Wilson *et al.*, 1995; Jourdan & Stauffer, 1998) (Fig. 1). Binding sites 2 and 3 are required for activation of the operon in response to glycine, whereas all three sites are required for repression in response to purines (Wilson *et al.*, 1995; Wonderling *et al.*, 2000).

GcvA-mediated regulation of *gcv* expression requires two additional proteins, Lrp and GcvR. Lrp, a global transcriptional regulator of genes involved in amino acid metabolism (Lin *et al.*, 1992; Calvo & Matthews, 1994), is required for both activation and repression of *gcv* expression (Stauffer & Stauffer, 1994). It has been

proposed that Lrp plays a structural role in *gcv* regulation, binding to and bending the DNA between GcvA-binding sites 1 and 2 to facilitate the formation of appropriate regulatory complexes (Stauffer & Stauffer, 1999) (Fig. 1).

GcvR, a negative-acting protein required for GcvA-mediated repression of *gcv* transcription (Ghrist & Stauffer, 1995), does not appear to regulate *gcv* expression directly, as it fails to bind to the *gcv* control region in gel mobility shift assays (A. Ghrist, unpublished results). In addition, GcvR does not regulate *gcv* expression indirectly by regulating the expression of *gcvA* or *gcvR* (Ghrist & Stauffer, 1998). Rather, repression of the *gcv* operon depends on the relative levels of expression of *gcvA* and *gcvR*; overexpression of *gcvA* results in *gcv* activation, whereas overexpression of *gcvR* results in super-repression (Ghrist & Stauffer, 1995). In light of these observations, we hypothesized that GcvR interacts directly with GcvA to control activation and repression of the *gcv* operon (Ghrist & Stauffer, 1998). In this study, we present evidence that supports this hypothesis. Using a LexA-based system for analysing protein heterodimerization, we show that GcvA and GcvR interact *in vivo*. In addition, we show that *gcvA* and *gcvR* mutations which decrease *gcv* repression also decrease heterodimerization.

METHODS

Bacterial strains and plasmids. All strains used in this study were constructed in our laboratory and are derivatives of the *E. coli* K-12 strain GS162 [*pheA905 thi Δ(argF–lac)U169 araD129 rpsL150 relA1 deoC1 flbB5301 ptsF25 rbsR*]. Ad-

Abbreviations: AP, ampicillin; DBD, DNA-binding domain; *gcv*, *gcvTHP*; TC, tetracycline; TPEG, phenylethyl-β-D-thiogalactoside.

Fig. 1. *gcv* control region. The GcvA upstream (sites 3 and 2) and downstream (site 1) binding sites (Wilson *et al.*, 1995) and the Lrp-binding region (Stauffer & Stauffer, 1994) are shown relative to the *gcv* promoter -35 , -10 and $+1$ sequences (Stauffer *et al.*, 1993). Not to scale.

ditional strain designations and mutations are as follows: GS1111, $\Delta gcvR$ *bcp* Σ neo; GS1118, $\Delta gcvA$ *orf2* Σ aadA; GS1131, $\Delta gcvR$ *bcp* Σ neo, $\Delta gcvA$ *orf2* Σ aadA. All lysogens denoted with $\lambda gcvT::lacZ$ carry a single chromosomal copy of a *gcvT::lacZ* reporter fusion in phage λ gt2 (Stauffer *et al.*, 1993). The lysogen GS1131 λ sulA::lacZ carries a single chromosomal copy of the *sulA::lacZ* reporter fusion from strain SU202 (Dmitrova *et al.*, 1998).

The multi-copy plasmid used in the *gcvR* mutant screen was pBR322 (Bolivar *et al.*, 1977). The low-copy-number plasmid used in this study was pSC101 (Cohen & Chang, 1977) and the single-copy plasmid used was pGS311 (Ghrist & Stauffer, 1995).

Media and growth conditions. The rich medium used was Luria–Bertani broth (LB) (Miller, 1992). The minimal media used were minimal salts (Vogel & Bonner, 1956) supplemented with phenylalanine ($50 \mu\text{g ml}^{-1}$), thiamin ($1 \mu\text{g ml}^{-1}$), and either 0.4% glucose (GM) or 0.4% lactose (LM). Agar was added at 1.5% to make solid media. Additional supplements were added, where indicated, at the following concentrations: inosine, $50 \mu\text{g ml}^{-1}$; glycine, $300 \mu\text{g ml}^{-1}$; phenylethyl- β -D-thiogalactoside (TPEG), 2 mM; ampicillin (AP), $150 \mu\text{g ml}^{-1}$ for multi-copy plasmids and $30 \mu\text{g ml}^{-1}$ for single-copy plasmids; tetracycline (TC), $10 \mu\text{g ml}^{-1}$. $\lambda gcvT::lacZ$ lysogens carry the ϵ 857 mutation resulting in a temperature-sensitive repressor and were grown at 30°C . All other strains were grown at 37°C .

Construction of LexA fusion plasmids. Wild-type and mutant alleles of *gcvA* were cloned into pMS604 (Dmitrova *et al.*, 1998) on PCR-generated *Bst*EII–*Xho*I fragments, creating in-frame fusions with the LexA DNA-binding domain (DBD). Wild-type and mutant *gcvR* alleles were cloned into pDP804 (Dmitrova *et al.*, 1998) on PCR-generated *Bss*HII–*Bgl*II fragments, creating in-frame fusions with the LexA408 DBD variant. The nucleotide sequence of each PCR-generated fragment was verified by DNA sequence analysis.

β -Galactosidase assays. β -Galactosidase assays were performed as described by Miller (1992). Each experiment was repeated at least twice, with each sample assayed in triplicate.

RESULTS

GcvR interacts with GcvA *in vivo*

To determine if GcvR interacts with GcvA *in vivo*, we used a LexA-based system for analysing protein heterodimerization developed for use in *E. coli* (Dmitrova *et al.*, 1998). In this system, the reporter strain carries a λ sulA::lacZ fusion under the control of a hybrid LexA operator containing a wild-type half-site and a mutant half-site (Fig. 2). The wild-type half-site is recognized by the wild-type LexA DBD and the mutant half-site is recognized by the LexA408 DBD variant. Interaction between protein domains fused to these LexA DBDs will

create LexA/LexA408 heterodimers capable of repressing the λ sulA::lacZ reporter (Fig. 2). For this experiment, *gcvA* was fused downstream of the wild-type LexA DBD sequence of pMS604 and *gcvR* was fused downstream of the LexA408 DBD sequence of pDP804. The expression of both fusion proteins is under the control of a *lacUV5* promoter with a down mutation in the ribosome-binding site. Since the reporter strain GS1131 λ sulA::lacZ carries a *lacI* deletion, both fusion proteins are constitutively expressed in this strain. The reporter strain transformed with one or both of these fusion plasmids was grown in LB (plus appropriate antibiotics) and β -galactosidase activity was determined. Expression of either fusion protein alone resulted in a small decrease in β -galactosidase activity (less than 30%) (Table 1). However, co-expression of both fusion proteins resulted in a significant decrease in β -galactosidase activity (Table 1, row 4). Because plasmids pMS604 and pDP804 are high-copy number and since each fusion protein alone had a small effect on *sulA::lacZ* expression, we tested whether the effects of the GcvA and GcvR fusion proteins alone were additive and partly responsible for some of the reduced expression of the *sulA::lacZ* fusion in the co-expression experiment. The wild-type *gcvA* gene fused to the LexA DBD in pMS604 was cloned into the low-copy-number plasmid pSC101. Expression of the fusion protein from the low-copy-number plasmid did not cause a decrease in β -galactosidase activity (Table 1, compare rows 1 and 10). When co-expressed with the GcvR fusion protein, there was still a significant reduction in β -galactosidase levels (Table 1, compare rows 1 and 11). These results indicate that GcvA and GcvR interact to form heterodimers *in vivo*.

Isolation of *gcvR* mutants unable to properly repress the *gcv* operon

To determine if *gcvR* mutants unable to properly repress *gcv* expression might also be defective in their ability to interact with GcvA, the following strategy was used to isolate *gcvR* mutants impaired in their ability to negatively regulate the *gcv* operon. The wild-type lysogen GS162 λ gcvT::lacZ, which carries a chromosomal *gcvT::lacZ* reporter fusion, was transformed with a multi-copy plasmid pool carrying PCR-induced random base pair changes in *gcvR*. Transformants were selected on LB+AP plates and then transferred to LM+AP plates supplemented with the purine nucleoside inosine and TPEG (an inhibitor of β -galactosidase); this medium results in maximal repression of

Fig. 2. Transcriptional repression by LexA heterodimer formation of GcvA and GcvR. The *sulA* -35 and -10 promoter elements are indicated by a line between the sequences and the mutant 408 and wild-type (wt) operator half-sites are in lower case (Dmitrova et al., 1998).

Table 1. Heterodimerization of GcvA and GcvR and their mutants

<i>gcvA</i> allele*	<i>gcvR</i> allele†	β -Galactosidase activity‡
None	None	1133 $\pm$ 145
None	<i>gcvR</i>	992 $\pm$ 34
<i>gcvA</i>	None	930 $\pm$ 5
<i>gcvA</i>	<i>gcvR</i>	109 $\pm$ 14
<i>gcvA</i>	<i>gcvRR129H</i>	548 $\pm$ 3
<i>gcvA</i>	<i>gcvRQ144P</i>	484 $\pm$ 20
<i>gcvAC169R</i>	<i>gcvR</i>	286 $\pm$ 46
<i>gcvAR197G</i>	<i>gcvR</i>	493 $\pm$ 23
<i>gcvA</i> Δ 292–305	<i>gcvR</i>	687 $\pm$ 16
<i>gcvA</i> §	None	1157 $\pm$ 71
<i>gcvA</i> §	<i>gcvR</i>	176 $\pm$ 40

* *gcvA* and its mutant alleles were fused in-frame to the *lexA* region of pMS604 (or pSC101§).

† *gcvR* and its mutant alleles were fused in-frame to the *lexA*408 region of pDP804.

‡ GS1131 λ *sulA*::*lacZ* transformants were grown in LB plus appropriate antibiotics and assayed for β -galactosidase activity. Activity is expressed in Miller units (Miller, 1992).

the *gcvT*::*lacZ* fusion and the inability to grow with lactose as the carbon source. Assuming that GcvR acts as a dimer (G. Heil, unpublished results), we reasoned that transformants expressing mutant GcvR proteins that could not interact with GcvA but that could still dimerize with wild-type GcvR and interfere with its ability to repress *gcv* expression would be able to grow on the LM plates due to increased β -galactosidase activity. Five transformants with this phenotype were isolated. Plasmid DNA was purified from each of these transformants and used to transform the Δ *gcvR* strain GS1111 λ *gcvT*::*lacZ*. The resulting transformants were grown in GM supplemented with inosine and assayed for β -galactosidase activity. All five were found to have increased β -galactosidase activity as compared to GS1111 λ *gcvT*::*lacZ* transformed with a multi-copy plasmid carrying the wild-type *gcvR* allele, indicating that each encoded a GcvR protein defective in negative regulation of the *gcv* operon (data not shown).

Each of the mutant *gcvR* alleles was sequenced and the following mutations were identified: *gcvR* Δ 99–189 and *gcvR* Δ 131–189 contain stop codons at positions 99 and 131, respectively; *gcvR* Δ 106 contains a single nucleotide

Table 2. *gcvA* and *gcvR* mutations and their effects on *gcvT*::*lacZ* expression

Strain*	Plasmid-encoded <i>gcvA</i> or <i>gcvR</i> allele†	β -Galactosidase activity‡ in cells grown in:§		
		GM	Glycine	Inosine
Wild-type	None	233	1395	22
Δ <i>gcvR</i>	None	1756	2122	1209
Δ <i>gcvR</i>	<i>gcvR</i> Δ 131–189	2041	2448	1340
Δ <i>gcvR</i>	<i>gcvR</i> Δ 106	2070	2561	1435
Δ <i>gcvR</i>	<i>gcvR</i> Δ 99–189	1863	2060	1111
Δ <i>gcvR</i>	<i>gcvRR129H</i>	1156	1891	715
Δ <i>gcvR</i>	<i>gcvRQ144P</i>	2011	1767	1362
Δ <i>gcvA</i>	None	110	127	54
Δ <i>gcvA</i>	<i>gcvAC169R</i>	529	1006	261
Δ <i>gcvA</i>	<i>gcvAR197G</i>	1522	2373	1066
Δ <i>gcvA</i>	<i>gcvA</i> Δ 292–305	1413	2026	1070

* Transformants of GS162 λ *gcvT*::*lacZ* (wild-type), GS1111 λ *gcvT*::*lacZ* (Δ *gcvR*) or GS1118 λ *gcvT*::*lacZ* (Δ *gcvA*).

† All mutant alleles were expressed from the single-copy plasmid pGS311.

‡ Activity is expressed in Miller units (Miller, 1992). All assays were repeated at least twice. Data from a representative experiment are shown.

§ Transformants were grown in GM + AP with supplements as indicated and assayed for β -galactosidase activity.

deletion (C) at codon 106, resulting in a reading frameshift and a stop codon at position 108, producing a protein that is 107 amino acids in size with two missense amino acids at its C-terminus; *gcvRR129H* contains an arginine to histidine change at position 129; and *gcvRQ144P* contains a glutamine to proline change at position 144.

To determine the effects of these mutant alleles on *gcv* expression when they are expressed at wild-type levels, each mutant *gcvR* allele was cloned into the single-copy plasmid pGS311. The resulting single-copy constructs were used to transform the Δ *gcvR* strain GS1111 λ *gcvT*::*lacZ*. Transformants were grown in GM, GM + glycine and GM + inosine media and assayed for β -galactosidase activity (Table 2). The three deletion alleles as well as *gcvRQ144P* led to a complete loss of *gcv* repression in all three media, exhibiting β -galactosidase activities nearly identical to the levels observed in the Δ *gcvR* control. The *gcvRR129H* allele

Fig. 3. (a) The 189 amino acid GcvR protein and the GcvR fragments fused to the LexA87 408 headpiece in plasmid pDP804 used to define regions of GcvR involved in interacting with GcvA. The R129 and Q144 amino acids involved in negative regulation are indicated relative to the deletion end points. (b) The 305 amino acid GcvA protein and the GcvA fragments fused to the LexA87 wild-type headpiece in plasmid pMS604 used to define the region of GcvA involved in interacting with GcvR. The C169 and R197 amino acids and the C-terminal 14 amino acids involved in negative regulation are indicated. H-T-H, helix-turn-helix domain. Not to scale.

also led to a loss of repression, but the phenotype was less severe. It should be noted that the 1.5–2-fold repression observed in GM + inosine medium is due to the PurR repressor, which has been shown to repress transcription of the *gcv* operon independently of GcvA/GcvR-mediated repression (Wilson *et al.*, 1993a).

GcvR mutants unable to repress *gcv* expression are impaired in their ability to interact with GcvA

If GcvA interacts directly with GcvR to regulate the *gcv* operon, one possible explanation for the loss of repression displayed by the *gcvR* mutants described above is that they are unable to interact with GcvA. Therefore, the *gcvR*Q144P and *gcvR*R129H alleles were cloned into pDP804 and tested for their ability to interact with GcvA using the protein heterodimerization system. Both mutant alleles were impaired in their ability to interact with GcvA, displaying an approximately 4.5–5.5-fold increase in expression of the *λsulA::lacZ* reporter fusion as compared to the wild-type *gcvR* allele (Table 1, compare row 4 with rows 5 and 6).

Deletion analysis of *gcvR*

To define the region(s) of GcvR involved in its interaction with GcvA, a 3' deletion (*gcvR*Δ143–189) and a 5' deletion (*gcvR*Δ1–35) of *gcvR* (Fig. 3a) were cloned

Table 3. Deletions of *gcvA* and *gcvR* and their effects on GcvA/GcvR interaction

<i>gcvA</i> allele*	<i>gcvR</i> allele†	β-Galactosidase activity‡
None	None	1195 ± 165
None	<i>gcvR</i>	1102 ± 96
<i>gcvA</i>	None	999 ± 79
<i>gcvA</i>	<i>gcvR</i> Δ143–189	711 ± 2
<i>gcvA</i>	<i>gcvR</i> Δ1–35	949 ± 129
<i>gcvA</i> Δ1–151	<i>gcvR</i>	143 ± 13
<i>gcvA</i> Δ1–230	<i>gcvR</i>	821 ± 18
<i>gcvA</i> Δ1–151·Δ231–305	<i>gcvR</i>	1076 ± 108

* *gcvA* and its mutant alleles were fused in-frame to the *lexA* region of pMS604.

† *gcvR* and its mutant alleles were fused in-frame to the *lexA*408 region of pDP804.

‡ GS1131 λ *sulA::lacZ* transformants were grown in LB plus appropriate antibiotics and assayed for β-galactosidase activity. Activity is expressed in Miller units (Miller, 1992).

into pDP804 and tested for their ability to interact with GcvA using the LexA heterodimerization assay. Since all of the *gcvR* mutations described above were located in the 3' half of *gcvR*, it was not surprising that deletion of the C-terminal 47 residues of GcvR, which includes the glutamine at position 144, resulted in a loss of interaction with GcvA (Table 3, row 4). It was somewhat surprising, however, that deletion of the N-terminal 35 residues also resulted in a loss of interaction with GcvA (Table 3, row 5).

Isolation of GcvA mutants refractory to inhibition by GcvR

In a previous study, two *gcvA* mutants that are able to activate but not repress *gcv* expression were isolated but not characterized (Jourdan & Stauffer, 1998). In that same study, a deletion of the sequence encoding the C-terminal 14 amino acids of GcvA (Δ *gcvA*944; renamed *gcvA*Δ292–305 in this study) (Fig. 3b) was constructed and shown to have the same phenotype. During this study, the uncharacterized *gcvA* mutant alleles were sequenced and the mutations were identified as a cysteine to arginine change at position 169 (*gcvA*C169R) and an arginine to glycine change at position 197 (*gcvA*R197G) (Fig. 3b). To measure the effects of these mutations on *gcv* expression, they were cloned into the single-copy plasmid pGS11 and the resulting constructs were used to transform the Δ *gcvA* reporter strain GS1118 λ *gcvT::lacZ*. Transformants were grown in GM, GM + glycine and GM + inosine media and assayed for β-galactosidase activity. As expected, the Δ *gcvA* control lysogen showed no significant change in β-galactosidase activity under any growth condition, except for the twofold PurR effect (Table 2). The *gcvA*R197G and *gcvA*Δ292–305 mutations resulted in a complete loss of *gcv* repression, producing levels of β-

galactosidase activity similar to that observed in the $\Delta gcvR$ control (Table 2, rows 10 and 11). The *gcvAC169R* mutation also led to a loss of *gcv* repression, although the phenotype was not as severe (Table 2, row 9).

GcvA mutants unable to repress *gcv* expression are impaired in their ability to interact with GcvR

To test whether the *gcvA* mutants described above are defective in their ability to interact with GcvR, each mutant allele was cloned into pMS604 and its interaction with GcvR measured using the LexA heterodimerization system. The *gcvAC169R* mutation resulted in a small loss of interaction with GcvR, whilst the *gcvAR197G* and *gcvA Δ 292–305* mutations resulted in more severe losses of interaction with GcvR (Table 1, compare row 4 with rows 7, 8 and 9). These results are consistent with the effects of the mutations on repression of the *gcv* operon.

The C-terminal half of GcvA is able to interact with GcvR

To define the region(s) of GcvA that is involved in its interaction with GcvR, several 5' and 3' deletions of *gcvA* (*gcvA Δ 1–151*, *gcvA Δ 1–230* and *gcvA Δ 1–151 $\cdot\Delta$ 231–305*) (Fig. 3b) were cloned into pMS604 and tested for their ability to interact with GcvR using the protein heterodimerization system. Deletion of the N-terminal 151 amino acids of GcvA had no effect on its ability to interact with GcvR (Table 3, row 6). However, deletion of the N-terminal 230 amino acids, which deletes the cysteine at position 169 and the arginine at position 197, or deletion of amino acids 1–151 and 231–305, resulted in significant loss of interaction with GcvR (Table 3, rows 7 and 8). These results suggest that the GcvA/GcvR interaction requires the C-terminal half of GcvA.

The C-terminal half of GcvA interferes with inhibition of wild-type GcvA by GcvR

Sequence and mutational analysis indicate that the N-terminal half of GcvA is involved in DNA binding and activation (Wilson & Stauffer, 1994; Jourdan & Stauffer, 1998) and results from this study suggest that the C-terminal half of GcvA is involved in an interaction with GcvR. To determine if overexpression of the C-terminal half of GcvA would lead to activation of the *gcv* operon by interfering with the ability of GcvR to inhibit wild-type GcvA, the wild-type strain GS162 $\lambda gcvT::lacZ$ and the $\Delta gcvA$ strain GS1118 $\lambda gcvT::lacZ$ were transformed with plasmids carrying the *lexA-gcvA Δ 1–151* and *lexA-gcvA Δ 1–230* alleles used in the heterodimerization experiment. Transformants were grown in GM, GM+glycine and GM+inosine media and assayed for β -galactosidase activity. Transformation of the $\Delta gcvA$ strain GS1118 $\lambda gcvT::lacZ$ with either plasmid resulted in low non-inducible *gcv* expression, identical to that observed

Table 4. LexA-GcvA Δ 1–151 interferes with inhibition of wild-type GcvA by GcvR

<i>gcvA</i> allele*	β -Galactosidase activity† in cells grown in:‡		
	GM	Glycine	Inosine
None	135	817	21
<i>gcvAΔ1–151</i>	847	994	340
<i>gcvAΔ1–230</i>	165	914	27

**gcvA* deletion alleles were fused to the *lexA408* region of plasmid pMS604.

† Activity is expressed in Miller units (Miller, 1992). All assays were repeated at least twice. Data from a representative experiment are shown.

‡ GS162 $\lambda gcvT::lacZ$ transformants were grown in GM+TC medium with supplements as indicated and assayed for β -galactosidase activity.

in the untransformed control (data not shown). Thus, neither LexA- Δ GcvA protein fusion is able to activate the *gcvT::lacZ* fusion. Transformation of wild-type strain GS162 $\lambda gcvT::lacZ$ resulted in normal regulation of *gcv* expression in the case of the *lexA-gcvA Δ 1–230* allele, whereas transformation with the *lexA-gcvA Δ 1–151* allele led to constitutive activation in all three media (Table 4).

DISCUSSION

In this report, we present evidence to support a model in which GcvR interacts with GcvA to prevent activation of the *gcv* operon. Using a LexA-based system for measuring protein heterodimerization, we were able to show that GcvA and GcvR interact *in vivo*. In addition, mutations in both *gcvA* and *gcvR* which result in a loss of *gcv* repression also result in a loss of GcvA/GcvR interaction. Thus, there is a strong correlation between the ability of these proteins to form heterodimers and their ability to repress expression of the *gcv* operon.

Three lines of evidence suggest that the C-terminal half of GcvA is involved in heterodimerization with GcvR. First, substitution mutants of GcvA which result in a loss of interaction with GcvR lie in the C-terminal half of GcvA. Second, deletion of the N-terminal half of GcvA has no effect on its ability to interact with GcvR, whereas deletion of the C-terminal 14 amino acids results in a complete loss of heterodimerization. Finally, although unable to activate *gcv* transcription, the C-terminal half of GcvA is able to interfere with GcvR-mediated repression of wild-type GcvA, probably by titrating the available antiactivator, leading to constitutive activation of the operon (Table 4). Together, these results suggest that the C-terminal half of GcvA is involved in an interaction with GcvR.

All of the *gcvR* mutations identified in this study that result in a decrease in repression of the *gcv* operon as

well as a decrease in heterodimerization lie in the C-terminal half of the protein, suggesting that this region is involved in an interaction with GcvA. Consistent with these results, a deletion of the C-terminal 47 amino acids resulted in a loss of interaction with GcvA (Table 3). However, deletion of the N-terminal 35 amino acids also resulted in a loss of heterodimerization (Table 3). Since our screen relied on the ability of mutant GcvR to interact with wild-type GcvR, it is possible that mutations in the N-terminal region that would have resulted in a loss of GcvR interaction with GcvA were excluded from the screen as the mutations might have also resulted in a loss of GcvR homodimerization. It is also possible that deletion of this region of *gcvR* results in a decrease in expression or protein stability.

While it is clear that GcvR interacts with GcvA to repress the *gcv* operon, it is not clear how this interaction is modified by glycine and purines and, in turn, how this modification leads to the correct regulation of *gcv* expression. In previous studies, we have shown that neither glycine nor purines have an effect on the expression of *gcvA* or *gcvR* (Wilson & Stauffer, 1994; Ghrist & Stauffer, 1998). These results led us to propose a model in which GcvA homocomplexes function as activators, whilst GcvA/GcvR heterocomplexes function as repressors. In this model, the coeffectors influence the type of complex formed; glycine promotes the formation of activation complexes, whilst purines promote the formation of repression complexes. Increasing the expression of GcvA or GcvR would force the formation of activation or repression complexes, respectively. We used the LexA-based heterodimerization system to measure the effects of glycine and inosine on the ability of GcvA and GcvR to interact by growing transformants in GM, GM + glycine and GM + inosine media prior to assaying for β -galactosidase activity. However, glycine and inosine had no significant effect on the GcvA/GcvR interaction (data not shown). This may be due to the high level of expression of both fusion proteins in this assay and *in vitro* studies with purified GcvA and GcvR will be needed to provide further data to prove or disprove this model.

Regulation of the *gcv* operon by GcvA and GcvR is part of a growing number of prokaryotic regulatory systems involving an activator/antiactivator complex, including TraR/TraM regulation of conjugal transfer of the Ti plasmid in *Agrobacterium tumefaciens* (Hwang *et al.*, 1999), NifA/NifL regulation of nitrogen fixation in *Klebsiella pneumoniae* (Narberhaus *et al.*, 1995), ComK/MecA/ClpC regulation of competence in *Bacillus subtilis* (Turgay *et al.*, 1998) and CRP/CytR regulation of operons encoding the nucleoside transport and biosynthesis enzymes (Valentin-Hansen *et al.*, 1996). In the case of TraR/TraM, NifA/NifL and ComK/MecA/ClpC, the activator is sequestered by the anti-activator(s), thus preventing binding and activation. In the case of CRP/CytR, the antiactivator binds DNA between activator-binding sites, forming a nucleoprotein complex which prevents activation (Valentin-Hansen *et al.*, 1996). Evidence suggests that GcvR could

act in both ways to negatively regulate *gcv* expression. As discussed above, the C-terminal half of GcvA, deleted for the DNA-binding region (Jourdan & Stauffer, 1998), prevents GcvR-mediated repression. This suggests that GcvR may function by sequestering GcvA in an inactive form. On the other hand, overexpressing GcvR in a *gcvA*⁺ strain results in a lower level of *gcv* expression than that observed in a *gcvA* mutant (Ghrist & Stauffer, 1995). In addition, GcvA/GcvR, in the presence of inosine, repress a *gcvT*::*lacZ* fusion four- to fivefold in a GcvA-binding site 1-dependent manner (Wonderling *et al.*, 2000). These results suggest that GcvA and GcvR act together at the *gcv* control region to directly repress *gcv* expression.

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