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Positive Regulation of the *Escherichia coli* Glycine Cleavage Enzyme System

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A new mutation in *Escherichia coli*, designated *gcvA1*, that results in noninducible expression of both *gcv* and a *gcvT-lacZ* gene fusion was isolated. A plasmid carrying the wild-type *gcvA* gene complemented the mutation and restored glycine-inducible *gcv* and *gcvT-lacZ* gene expression. These results suggest that *gcvA* encodes a positive-acting regulatory protein that acts in *trans* to increase expression of *gcv*.

In enteric bacteria, the conversion of serine to glycine and 5,10-methylenetetrahydrofolate occurs through the action of the enzyme serine hydroxymethyltransferase, the *glyA* gene product (10). This reaction is an important contributor of one-carbon units in cell metabolism. The oxidative cleavage of glycine by the glycine cleavage (GCV) enzyme system provides a second pathway for one-carbon biosynthesis (14). A glycine-inducible GCV enzyme system has been demonstrated in both *Escherichia coli* (8, 11, 12) and *Salmonella typhimurium* (16).

E. coli mutants blocked simultaneously in the GCV enzyme system and in the serine biosynthetic pathway are unable to use glycine as a serine source and require an exogenous source of serine (the GCV$^-$ phenotype). At present, six classes of mutations have been shown to result in the GCV$^-$ phenotype under the appropriate growth conditions. The first class maps at min 62.6 on the *E. coli* chromosome and presumably affects the *gcv* structural genes (12). The second class maps at min 14.8 and disrupts the lipoic acid biosynthetic pathway (18). The third class maps at min 2.7 and alters the *lpd* gene, encoding the L protein of the GCV enzyme complex (17). The fourth class maps at min 54.8 and partially inactivates serine hydroxymethyltransferase, the *glyA* gene product (13). The fifth class maps at min 95.6 and disrupts the *cycA* gene involved in glycine transport (2, 4); this class results in the GCV$^-$ phenotype because of altered glycine uptake (4a). The sixth class maps at min 20 and inactivates the *lrp* gene (7). We report here a seventh locus that results in a GCV$^-$ phenotype because of the cell's inability to induce *gcv* expression.

Isolation of a new class of *gcv* mutations. Using a penicillin counterselection previously described (1, 11, 12), we isolated several mutants defective in the GCV enzyme pathway. One of the mutants isolated that displayed the GCV$^-$ phenotype and that had a very low level of GCV enzyme activity did not map with the known structural genes encoding GCV. This strain was designated GS786 (the complete genotypes of all strains used in this study are listed in Table 1). A [2-^{14}C]glycine uptake assay was performed to determine whether transport was altered in strain GS786. This strain was found to have normal transport of glycine (data not shown). Mutations in lipoic acid biosynthesis also result in a GCV$^-$ phenotype in serine auxotrophs, and growth of these mutants on glucose minimal medium (GM) containing glycine can be restored if lipoic acid is added to the medium (18). However, the addition of exogenous lipoic acid did not restore the GCV$^+$ phenotype in strain GS786. These results suggest that the mutation in GS786 represents a new class of *gcv* mutations, designated *gcvA1*.

Mapping the *gcvA1* mutation. To map the *gcvA1* mutation we used the comprehensive mapping kit for *E. coli* developed by Singer et al. (15). An interrupted mating experiment localized *gcvA1* between min 59 and min 69 on the *E. coli* chromosome. A P1 transduction analysis was then used to map the mutation more precisely (9). P1 *clr-100* phage lysates were prepared on donor strains that carry Tn*10* elements at various locations in the 60- to 70-min region of the *E. coli* chromosome. The phage lysates were then used in transductions with the *gcvA1* strain GS786 as the recipient. Transductants were selected on Luria agar (9) supplemented with tetracycline (10 μg/ml), and tetracycline-resistant (Tc^r) transductants were then scored on GM (19) plates supplemented with either glycine (300 μg/ml) or serine (200 μg/ml), as well as additional supplements as required. When strains carrying Tn*10* elements at min 68.7, 66.4, 65.0, or 62.2 were used as donors, all Tc^r transductants were unable to grow on glycine-supplemented plates (0 of 95 for each transduction). When NK5992 (*argA81*::Tn*10* at min 60.5) was used as the donor, 66% of the Tc^r transductants (40 of 61) were also able to grow on glycine-supplemented plates. The cotransduction frequency between *gcvA1* and *argA81*::Tn*10* was converted to distance (20), with 2.0 min of the *E. coli* chromosome being the maximum amount carried by P1 phage (2). Since the donor strain carried the Tn*10* transposon, 0.25 min was

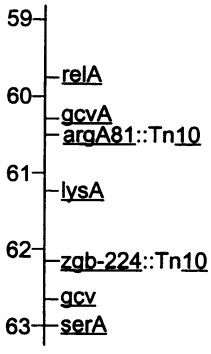

FIG. 1. Segment of the *E. coli* linkage map from min 59 to 63. For clarity, several genes known to map in this region of the chromosome are not included.

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TABLE 1. Bacterial strains used in this study

Strain	Genotype		Source
	Relevant	Other	
GS162	+ (wild type)	*thi pheA905 ΔlacU169 araD129 rpsL150*	This laboratory
GS786	*serA25 gcvA1*	*thi-1 lacY1 gal-6 malA1 lysA xyl-7 mtl-2 rpsL133 tonA2* λ^r λ^-	This laboratory
GS958	*serA25*	*thi pheA905 ΔlacU169 araD129 rpsL150*	This laboratory
GS970[a]	*serA25 gcvA1*	*thi pheA905 ΔlacU169 araD129 rpsL150*	This laboratory
GS973[b]	*gcvA1*	*thi pheA905 ΔlacU169 araD129 rpsL150 λgcvT-lacZ*	This laboratory
NK5992	*argA81*::Tn*10*	IN(*rrnD-rrnE*)*1*	B. Bachmann

[a] To construct strain GS970, the *gcvA1* allele was transferred into the *serA25* strain GS958 by using P1 transduction with the closely linked *argA81*::Tn*10* marker and then scoring transductants for the GCV$^-$ phenotype; the *argA81*::Tn*10* marker was finally removed by a second transduction.

[b] To construct strain GS973, the *gcvA1* allele was transferred into lysogen GS162λ*gcvT-lacZ* by using P1 transduction with the closely linked *argA81*::Tn*10* marker and then scoring transductants for loss of activation of the λ*gcvT-lacZ* fusion; the *argA81*::Tn*10* marker was finally removed by a second transduction.

subtracted from the 2.0 min to account for the amount of the transducing particle that is the Tn*10* element. The *argA81*::Tn*10* and *gcvA1* markers are about 0.2 min apart. We could not determine from the transduction data on which side of the *argA81*::Tn*10* marker *gcvA1* maps.

Cloning the *gcvA* gene. To clone the *gcvA* gene, we used the miniset of specialized transducing bacteriophages of Kohara and collaborators (6). In brief, a series of these bacteriophages containing *E. coli* DNA covering the deduced map position of *gcvA* was used to infect GS970, a *serA25 gcvA1* strain. Infected cells were then plated on GM plates containing glycine and the appropriate supplements. Since GS970 is unable to use glycine as a serine source, only cells containing a functional *gcvA* gene from the λ bacteriophage should be able to grow. Two such bacteriophages, 10B6 and 9A12, complemented the GCV$^-$ phenotype and localized *gcvA1* to min 60.3 (Fig. 1). DNA was prepared from phage 9A12 and digested with *Eco*RI, and an 11.6-kb insert fragment that spans the region common to phages 10B6 and 9A12 was isolated. This fragment was ligated into the *Eco*RI site of the single-copy plasmid pGS225, a pDF41 derivative (5) containing the Tn*5 kan* gene (3). This new plasmid was designated pGS254.

We tested whether plasmid pGS254 could complement the GCV$^-$ phenotype by transforming strain GS970 with plasmid pGS254. Transformants were selected on Luria agar supplemented with kanamycin (20 μg/ml). When the Kn^r transformants were spotted on GM plates plus glycine, the plasmid was able to complement the GCV$^-$ phenotype, allowing growth in the presence of glycine.

TABLE 2. β-Galactosidase and GCV enzyme activities for *gcvA*$^+$ and *gcvA1* strains transformed with plasmid pGS254 or pGS225

Strain[a]	β-Galactosidase activity[b]		GCV enzyme sp act[c]	
	No glycine	Glycine	No glycine	Glycine
GS162λ*gcvT-lac*/pGS225 (*gcvA*$^+$)	179	763	0.04	1.24
GS973λ*gcvT-lac*/pGS225 (*gcvA1*)	111	117	0.02	0.02
GS162λ*gcvT-lac*/pGS254 (*gcvA*$^+$/*gcvA*$^+$)	177	718	0.06	0.96
GS973λ*gcvT-lac*/pGS254 (*gcvA1*/*gcvA*$^+$)	157	664	0.04	1.07

[a] The strains are isogenic except for the *gcvA* allele. Cells for enzyme assays were grown in GM medium without or with glycine.

[b] In Miller units (14).

[c] In nanomoles of HCHO generated per milligram of protein per minute.

β-Galactosidase and GCV enzyme activities for wild-type and *gcvA1* strains transformed with plasmid pGS254. We tested the effect of the *gcvA1* mutation in cells lysogenized with λ*gcvT-lacZ* phage, which carries an in-frame translational fusion of the *gcvT* gene to *lacZ* (16a). In this fusion, β-galactosidase synthesis is under control of the *gcv* regulatory region. Strain GS162 was lysogenized with λ*gcvT-lacZ* phage and isogenic strains GS162λ*gcvT-lacZ* (*gcvA*$^+$) and GS973λ*gcvT-lacZ* (*gcvA1*) were transformed with either pGS225 containing no insert DNA as a control or plasmid pGS254 carrying the wild-type *gcvA* gene. Strains GS973λ*gcvT-lacZ* and GS162λ*gcvT-lacZ*, with either plasmid pGS225 or plasmid pGS254, were then grown in GM medium without or with glycine and assayed for β-galactosidase and GCV enzyme activities. In the *gcvA*$^+$ lysogen GS162λ*gcvT-lacZ*/pGS225, the addition of glycine to the growth medium resulted in induction of both β-galactosidase and GCV enzyme activities (Table 2). In the *gcvA1* lysogen GS973λ*gcvT-lacZ*/pGS225, however, the addition of glycine to the growth medium had no effect on β-galactosidase or GCV enzyme activity. Plasmid pGS254 complemented the *gcvA1* mutation in lysogen GS973λ*gcvT-lacZ*, restoring both β-galactosidase and GCV enzyme activities (Table 2).

The *gcvA1* mutation shares a number of characteristics with mutations in other genes encoding positive-acting regulatory proteins. First, the *gcvA1* mutation lies outside the *gcv* structural genes and thus acts in *trans*. Second, the *gcvA1* mutation is recessive to the wild-type allele (Table 2). Finally, since the *gcvA1* mutation affects not only GCV levels but also β-galactosidase levels encoded by the λ*gcvT-lacZ* fusion, it is likely to affect synthesis of the gene products rather than their activities. These results suggest that plasmid pGS254 carries the *gcvA* gene and that *gcvA* encodes a positive-acting regulatory protein that acts in *trans* to activate expression of the *gcv* system. How this gene activates *gcv* expression and how *gcvA* itself is regulated are under study.

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