Role for the leucine-responsive regulatory protein (Lrp) as a structural protein in regulating the *Escherichia coli* *gcvTHP* operon

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The *Escherichia coli* glycine-cleavage enzyme system (*gcvTHP* and *lpd* gene products) provides C₁ units for cellular methylation reactions. Both the GcvA and leucine-responsive regulatory (Lrp) proteins are required for regulation of the *gcv* operon. One model proposed for *gcv* regulation is that Lrp plays a structural role, bending the DNA to allow GcvA to function as either an activator or a repressor in response to environmental signals. This hypothesis was tested by replacing all but the upstream 22 bp of the Lrp-binding region in a *gcvT::lacZ* fusion with the I1A site from phage λ . Integration host factor (IHF) binds the I1A site and bends the DNA about 140°. Shifting the I1A site by increments of 1 base around the DNA helix resulted in IHF-dependent activation and repression of *gcvT::lacZ* expression that were face-of-the-helix dependent. Activation was also dependent on the GcvA protein, and repression was dependent on both the GcvA and GcvR proteins, demonstrating that the roles for these proteins were not altered. The results are consistent with Lrp playing primarily a structural role in *gcv* regulation, although they do not completely rule out the possibility that Lrp also interacts with another *gcv*-regulatory protein or with RNA polymerase.

Keywords: *gcv* operon, GcvA, Lrp, IHF, DNA bending

INTRODUCTION

In *Escherichia coli*, serine hydroxymethyltransferase (*glyA* gene product) catalyses the conversion of serine to glycine and 5,10-methylenetetrahydrofolate (Mudd & Cantoni, 1964). The glycine-cleavage (GCV) enzyme system (*gcvTHP* and *lpd* gene products) oxidatively cleaves glycine into CO₂, NH₃ and 5,10-methylenetetrahydrofolate and provides a second pathway for one-carbon (C₁) units (Kikuchi, 1973). Glycine is used in protein and purine biosynthesis, and the C₁ units are used in the biosynthesis of purines, methionine, thymine and other cellular methylation reactions (Mudd & Cantoni, 1964). Strains defective in the GCV enzyme system excrete glycine (Plamann *et al.*, 1983; Ghrist & Stauffer, 1995b), and it is likely that the role for the GCV enzyme complex is to balance the cell's requirements for glycine and C₁ units used in these various biosynthetic and methylation reactions.

Multiple *gcv*-specific and global-acting regulatory

proteins are involved in regulation of *gcv* expression. The GcvA protein functions as both an activator and a repressor for the *gcv* operon (Wilson *et al.*, 1993a, b). Three sites were identified for GcvA binding in the *gcv* control region, from bases –271 to –242 (site 3), –241 to –214 (site 2), and from bp –69 to –34 (site 1) relative to the transcription-initiation site (Fig. 1a) (Wilson *et al.*, 1995). Binding sites 3 and 2 are required for GcvA-mediated activation of the operon in response to glycine, whereas all three sites are required for GcvA-mediated repression of the operon in response to purines (Wilson *et al.*, 1995). In the presence of both glycine and purines, activation of the operon occurs (Stauffer & Stauffer, 1994).

The GcvR protein also negatively regulates the *gcv* operon. A *gcvR* mutation results in constitutive expression of a *gcvT::lacZ* fusion, and overexpression of *gcvR* results in superrepression (Ghrist & Stauffer, 1995a). However, repression does not occur in a *gcvA* mutant even when *gcvR* is overexpressed, suggesting that the ability of GcvR to repress is dependent on GcvA. A direct role for GcvR in the mechanism of repression has not been demonstrated.

Abbreviations: GCV, glycine cleavage; GM, glucose minimal; IHF, integration host factor; RNAP, RNA polymerase.

Fig. 1. (a) Diagram showing the locations of the GcvA upstream (sites 3 and 2) and downstream (site 1) binding sites (Wilson *et al.*, 1995), the Lrp-binding region (Stauffer & Stauffer, 1994), and the PurR-binding site (Wilson *et al.*, 1993a) relative to one another and to the *gcv* promoter -35, -10 and +1 sequences (Stauffer *et al.*, 1993). The position of the PmeI and HindIII sites used to facilitate the replacement of the Lrp-binding region with the IHF-binding site I1A, and the XbaI sites generated by PCR to construct pGS466, are indicated. Plasmids pGS398 and pSX1 are described in Methods. (b) Diagram depicting the relative locations of the IHF-binding site around the surface of the DNA helix. The IHF-A insert was arbitrarily assigned its position, and the other inserts were assigned positions relative to the IHF-A insert.

PurR, the repressor for genes involved in nucleotide metabolism (Kilstrup *et al.*, 1989; Rolfes & Zalkin, 1988), mediates a twofold repression of the *gcv* operon in response to purine supplementation (Stauffer *et al.*, 1994; Wilson *et al.*, 1993a). PurR binds to a site overlapping the *gcv* operon transcription initiation site, from base -3 to +17 (Fig. 1a), and possibly interferes with RNA polymerase (RNAP) binding to the *gcv* promoter (Wilson *et al.*, 1993a). PurR-mediated repression occurs independently of the GcvA/GcvR system (Wilson *et al.*, 1993a; Ghrist & Stauffer, 1995a).

Lrp is a global regulator of numerous genes relating to amino acid metabolism and is known to bind DNA, bend it, and organize it into higher-order nucleoprotein structures (Calvo & Matthews, 1994; Newman *et al.*, 1996). Lrp is required for induction of the *gcv* operon (Lin *et al.*, 1992; Stauffer & Stauffer, 1994) and was shown to bind to multiple sites upstream of the *gcv* promoter, from about base -92 to -229 (Fig. 1a) (Stauffer & Stauffer, 1994). However, the mechanism of Lrp activation of the *gcv* operon is unknown.

Since GcvA-binding sites 3 and 2 are located a considerable distance upstream of the transcription initiation site (Fig. 1a), it was proposed that DNA looping is involved in the regulatory mechanism (Stauffer & Stauffer, 1998a, b). Lrp binds between GcvA-binding sites 3 and 2 and the *gcv* promoter, and it was proposed that an Lrp-induced bend enables GcvA bound at sites 3 and 2 to interact either with RNAP to activate the *gcv* promoter, or with GcvA bound at site 1 to repress the *gcv* operon. GcvR might function in this system to determine the protein-protein interactions that occur, or influence the angle or the degree of the DNA bend. Such protein-induced DNA bending is a general theme of gene regulation (van der Vliet & Verrijzer, 1993; Perez-Martin & de Lorenzo, 1997).

To assess whether Lrp is directly involved in activation of *gcv*, or whether an Lrp-induced change in the

structure of the DNA is required for transcriptional activation, we replaced the Lrp-binding region with the I1A site from phage λ for integration host factor (IHF) binding (Xin & Feiss, 1993). When IHF binds this site, it bends the DNA 140° (Xin & Feiss, 1993) and allowed us to test the contribution of protein-protein interactions against DNA curvature on the activation of transcription by Lrp. Our results are consistent with a model where Lrp introduces a bend in the DNA, and in this way either increases the probability of an interaction of the GcvA protein bound to sites 3 and 2 with RNAP bound to the *gcv* promoter or with GcvA bound at site 1, or possibly stabilizes these interactions.

METHODS

Strains and media. *E. coli* K-12 strains used in this study are listed in Table 1. The glucose-minimal (GM) medium used was the salts of Vogel & Bonner (1956) supplemented with

Table 1. *E. coli* strains used in this study

GS162 is a derivative of MC4100 (Casadaban, 1976). All other strains were constructed in this laboratory using GS162 as the parent strain. The *purR6::Tn10* allele is from strain S ϕ 5068 (Kilstrup *et al.*, 1989). The *lrp::Tn10* allele is from strain CV1008 (Platko *et al.*, 1990). The *gcvA1* (Wilson *et al.*, 1993b) and *gcvR::Tn10* (Ghrist & Stauffer, 1995) mutations were isolated in this laboratory. The *ihfB::Cm^r* allele is from strain MF1972 (Xu & Feiss, 1991) and was transduced into preexisting λ lysogens of strain GS162 as needed.

Strain	Relevant genotype	Other markers
GS162	Wild-type	<i>thi pheA905 ΔlacU169 araD129 rpsL150</i>
GS852	<i>purR6::Tn10</i>	As GS162
GS997	<i>lrp::Tn10</i>	As GS162
GS998	<i>gcvA1</i>	As GS162
GS1053	<i>gcvR::Tn10</i>	As GS162

0.4% glucose. Supplements were added at the following concentrations ($\mu\text{g ml}^{-1}$): glycine, 300; phenylalanine, 50; inosine, 50; thiamin, 1. GM medium was always supplemented with phenylalanine and thiamin since all strains carry the *pheA905* and *thi* mutations.

P1 transductions. P1 *cml clr*-100 phage was used for transductions as described by Miller (1992).

DNA manipulation. The procedures for plasmid DNA purification, restriction enzyme digestion, PCR reactions, etc. were as described by Sambrook *et al.* (1989). DNA sequencing was done using the Sequenase version 2.0 kit from USB.

Plasmid construction. Plasmid pGS318 is a derivative of pGS239 and carries an in-frame *gcvT::lacZ* gene fusion on a 769 bp *EcoRI*–*Bam*HI fragment (Stauffer *et al.*, 1993) with a unique *PmeI* site at bases –77 to –70 relative to the transcription initiation site (Stauffer & Stauffer, 1998a) (Fig. 1a). Using pGS318 as a template, bases –207T, –206T and –204A relative to the *gcv* transcription initiation site were changed to A, A and C, respectively, using the PCR megaprimer mutagenesis method (Sarkar & Sommer, 1990), generating a unique *Hind*III restriction site (Fig. 1a). The new plasmid was designated pGS398. pSX1 carries a 694 bp fragment from position –550 in the left end of the phage λ chromosome to bp +194 in the right end of the λ chromosome (Xu & Feiss, 1991). This fragment contains the IIA sequence shown to be an IHF-binding site (Xin & Feiss, 1993). pSX1 was used as template to PCR amplify the IIA sequence. The primers used flanked the IIA sequence and contained a *Hind*III site (upstream primer) and a *PmeI* site (downstream primer); when the PCR products are cut with *Hind*III and *PmeI* fragments of 133 bp are generated. The position of the IIA site, although located approximately in the centre, varied in each amplified fragment (Fig. 1b). The 133 bp *Hind*III–*PmeI* fragments carrying the IIA site were ligated into the *Hind*III/*PmeI* sites of pGS398, replacing a 133 bp region of pGS398 and all but the 5' 22 bp of the Lrp-binding sequence (Fig. 1a). The plasmids were designated pGS452(IHF-A) to pGS452(IHF-G) (see Fig. 1b), and the insertions were verified by DNA sequencing. pGS452(IHF-B) had an additional A to G transition mutation at base –118 relative to the transcription initiation site. This change is in the 133 bp λ phage sequence, but lies outside the IIA site, and the plasmid was not reconstructed. pGS466 carries the Lrp-binding region from base –278 to –37 on a 243 *Xba*I DNA fragment (Fig. 1a) cloned into the *Xba*I site of pBend2 (Kim *et al.*, 1989). The *Xba*I sites were generated by PCR.

Phage construction. The approximately 6 kb *EcoRI*–*MfeI* DNA fragments from pGS398 carrying the control *gcvT::lacZ* fusion and from pGS452 carrying each *gcvT::lacZ* fusion where the Lrp-binding region was replaced with the IHF-binding site were isolated and ligated into the *EcoRI* site of phage λ gt2 (Panasenkov *et al.*, 1977). The phages generated were single-plaque purified and designated λ *gcvT::lacZ*_{H-P}, λ *gcvT::lacZ*_{IHF-A}, λ *gcvT::lacZ*_{IHF-B}, λ *gcvT::lacZ*_{IHF-C}, λ *gcvT::lacZ*_{IHF-D}, λ *gcvT::lacZ*_{IHF-E}, λ *gcvT::lacZ*_{IHF-F} and λ *gcvT::lacZ*_{IHF-G}. The subscripts after *lacZ* indicate the control *Hind*III–*PmeI* fragment or the various fragments carrying the IHF-A to IHF-G replacements, respectively (Fig. 1b).

Construction of λ lysogens. λ lysogens were constructed as previously described (Urbanowski & Stauffer, 1986). All lysogens carry the *cI857* mutation resulting in a temperature-sensitive repressor and were grown at 30 °C. Lysogens were tested for the presence of a single copy of λ by assessing their

ability to support lytic infection by λ cI90c17 (Shimada *et al.*, 1972).

β -Galactosidase enzyme assays. β -Galactosidase enzyme activity was assayed from exponential-phase cultures harvested at an OD₆₀₀ of approximately 0.5 as described by Miller (1992) using the chloroform-SDS lysis procedure. All results are means from at least two separate assays in which the activity of each sample was determined in triplicate.

Gel-mobility shift assay. A double-stranded 769 bp *EcoRI*–*Bam*HI fragment carrying the *gcv* control region from pGS452(IHF-C) was ³²P-labelled at the *EcoRI* site, and a 373 bp *Bam*HI fragment from pGS466 was ³²P-labelled at the *Bam*HI sites using T4 polynucleotide kinase (Sambrook *et al.*, 1989). Less than 0.2 ng labelled DNA fragment was added to 20 μ l reaction mixtures containing DNA-binding buffer (10 mM Tris/HCl, pH 7.5, 50 mM KCl, 0.5 mM EDTA, 5% (v/v) glycerol, 1 mM DTT) and 125 μ g BSA ml⁻¹. The reaction mixtures were incubated at 37 °C for 5 min, 2 μ l purified Lrp was added to the mixtures as indicated in Fig. 2, and incubation was continued for an additional 15 min. One microlitre of loading dye (0.1% xylene cyanol and 50% glycerol) was added and the samples were immediately loaded onto a 5% polyacrylamide gel and run at approximately 12 V cm⁻¹. The gels were transferred to 3MM Whatman paper, dried and autoradiographed.

RESULTS AND DISCUSSION

Effects on *gcvT::lacZ* expression of replacing the Lrp-binding region with the IHF-binding site IIA from phage λ

Results from previous studies led to the hypothesis that Lrp, through its ability to bind to the *gcv*-control region and bend the DNA, played a structural role in regulation of the operon (Stauffer & Stauffer, 1998b). We tested this hypothesis by replacing the Lrp binding region in a *gcvT::lacZ* gene fusion with the phage λ IIA site; IHF binding at this site bends DNA approximately 140° (Xin & Feiss, 1993). In *E. coli*, a cAMP-receptor protein (CRP)-induced DNA bend that contributes to transcriptional activation can be replaced either by an intrinsically bent DNA fragment or by another protein-induced DNA bend (Bracco *et al.*, 1989; Gartenberg & Crothers, 1991; Dethiollaz *et al.*, 1996). Thus, we assumed that if the role for Lrp is to bend *gcv* DNA, replacing the Lrp-binding region with the IIA site would result in IHF-dependent regulation of *gcv*. Because the precise direction of the Lrp-induced bend relative to RNAP bound at the *gcv* promoter is unknown, and since there is a strict stereospecific requirement of GcvA bound at sites 3 and 2 for activation of a *gcvT::lacZ* fusion (Stauffer & Stauffer, 1998a, b), we constructed a series of insertions where the Lrp region was deleted and replaced with DNA of identical length containing the IIA site, but the position of the IIA site varied by 1 base in each construct (Fig. 1b; IHF-A to IHF-G). We assumed that in at least one construct, the IHF-induced bend would have GcvA at sites 3 and 2 phased appropriately with respect to RNAP. A λ gt2 phage carrying each insertion, as well as the control *gcvT::lacZ*_{H-P} fusion, was used to lysogenize the *purR*

strain GS852, in which inosine repression is due solely to the GcvA/GcvR system. The lysogens were grown in GM medium with the appropriate supplements, and β -galactosidase levels were measured. The control lysogen showed about a sixfold glycine-mediated activation and about a sevenfold inosine-mediated repression (Table 2). These results are similar to previous results with a wild-type *gcvT::lacZ* gene fusion (Stauffer & Stauffer, 1994), and show that the base changes introduced into the *gcv* control region in pGS398 to generate the *Hind*III and *Pme*I sites used in the construction of the λ *gcvT::lacZ*_{H-P} control phage and the I1A insertions do not significantly alter *gcvT::lacZ* expression.

The GS852 IHF-A lysogen showed about a 10-fold reduction in β -galactosidase levels when grown in GM medium (Table 2). Although glycine caused about a threefold induction, the induced levels were still 23-fold lower than in the control lysogen. The levels were not significantly repressed in cells grown in the presence of inosine. Moving the I1A site an additional 1 base around the DNA helix (IHF-B; Fig. 1b) increased the basal level of expression in GM significantly (Table 2). Glycine caused about a twofold induction, and the induced levels were only about sixfold lower than the control lysogen (Table 2). The levels were not significantly reduced in cells grown in the presence of inosine. When the I1A site was moved an additional 1 or 2 bases, in IHF-C and IHF-D, respectively, the basal levels of expression in GM increased to nearly the level observed in the control lysogen (Table 2). Glycine caused about a 3-fold and a 3.3-fold induction, respectively, and the induced levels were only about 2.5-fold lower than the level in the control lysogen. In addition, the levels were repressed about 2-fold in cells grown in the presence of inosine. Moving the I1A site an additional 1 or 2 bp, IHF-E and IHF-F, respectively, reduced the β -galactosidase levels under all growth conditions to the levels observed in the IHF-A lysogen (Table 2).

Table 2. Effects of replacing the Lrp-binding region with the λ IHF-binding site I1A on *gcvT::lacZ* expression in the *purR* strain GS852

Lysogens were grown in GM medium with the indicated supplements. All values were within 15% of the mean.

Lysogen	β -Galactosidase activity (Miller units)		
	None	Glycine	Inosine
852 λ <i>gcvT::lacZ</i> _{H-P}	131	786	18
852 λ <i>gcvT::lacZ</i> _{IHF-A}	13	34	11
852 λ <i>gcvT::lacZ</i> _{IHF-B}	69	134	49
852 λ <i>gcvT::lacZ</i> _{IHF-C}	108	308	60
852 λ <i>gcvT::lacZ</i> _{IHF-D}	91	302	45
852 λ <i>gcvT::lacZ</i> _{IHF-E}	20	33	17
852 λ <i>gcvT::lacZ</i> _{IHF-F}	14	26	10
852 λ <i>gcvT::lacZ</i> _{IHF-G}	2	13	<1

Since the GS852 IHF-C and IHF-D lysogens showed β -galactosidase levels that approached the levels measured in the control lysogen, we predicted that an insertion of the I1A site such that IHF would bind on the opposite face of the DNA helix relative to when bound to the IHF-C insertion would have the most severe effect on *gcvT::lacZ* expression. Thus, we constructed the insertion mutation IHF-G with the I1A site opposite that found in IHF-C (Fig. 1b). The insertion was cloned into λ gt2 phage, and the phage used to lysogenize strain GS852. The lysogen was grown in GM medium with the appropriate supplements, and β -galactosidase levels were measured. The GS852 IHF-G lysogen showed about a 65-fold reduction in β -galactosidase levels in GM medium compared to the control lysogen (Table 2). Although glycine caused an increase in β -galactosidase levels, the induced levels were 60-fold lower than the control lysogen. Moving GcvA-binding sites 3 and 2 by half integral turns of DNA was shown to result in a GcvA-mediated superrepression of a *gcvT::lacZ* fusion that was dependent on Lrp (Stauffer & Stauffer, 1998b). It is possible that an Lrp-induced bend results in GcvA bound on the wrong face of the DNA helix to make an inappropriate contact with the transcription apparatus, decreasing transcription of the operon. If this hypothesis is correct, it is likely that an IHF-induced bend in the GS852 IHF-G lysogen is responsible for the low levels of β -galactosidase measured in this lysogen.

Activation and repression are dependent on GcvA and GcvR

To determine whether the glycine-mediated activation and the inosine-mediated repression in the GS852 IHF-C and IHF-D lysogens and the superrepression in the IHF-G lysogen still required the GcvA and GcvR proteins, we lysogenized the *gcvA* strain GS998 and the *gcvR* strain GS1053 with phage carrying the IHF-C, IHF-D and IHF-G insertions, the lysogens were grown in GM medium with the appropriate supplements, and β -galactosidase levels were measured. The GS998 IHF-C and IHF-D lysogens showed β -galactosidase levels under all growth conditions that were increased compared to the 998 λ *gcvT::lacZ*_{H-P} control lysogen (Table 3). The levels were not induced by glycine or repressed by inosine, except for an expected twofold PurR-mediated repression. These results suggest that the glycine-mediated induction and the purine-mediated repression observed in the GS852 lysogens (see Table 2) are dependent on the GcvA protein. The GS998 IHF-G lysogen showed β -galactosidase levels that were about 4.5-fold lower than the GS998 control lysogen. These levels were not induced by glycine or repressed by inosine, except for the PurR-mediated repression. However, the levels were significantly increased compared to the levels observed in the GS852 IHF-G lysogen (compare Table 2, line 8, and Table 3, line 4). These results suggest that the reduced levels of expression in the GS852 IHF-G lysogen are due in part to increased GcvA-mediated repression that is relieved in the *gcvA* GS998 lysogen.

Table 3. Expression of *gcvT::lacZ* IHF lysogens in *gcvA*, *gcvR* and *lrp* strains

Lysogens were grown in GM medium with the indicated supplements. All values were within 11 % of the mean.

Lysogen	Relevant genotype	β -Galactosidase activity (Miller units)		
		None	Glycine	Inosine
998 λ <i>gcvT::lacZ</i> _{H-P}	<i>gcvA1</i>	75	76	39
998 λ <i>gcvT::lacZ</i> _{IHF-C}	<i>gcvA1</i>	99	104	56
998 λ <i>gcvT::lacZ</i> _{IHF-D}	<i>gcvA1</i>	121	122	69
998 λ <i>gcvT::lacZ</i> _{IHF-G}	<i>gcvA1</i>	17	17	8
1053 λ <i>gcvT::lacZ</i> _{H-P}	<i>gcvR::Tn10</i>	847	1197	781
1053 λ <i>gcvT::lacZ</i> _{IHF-C}	<i>gcvR::Tn10</i>	277	376	214
1053 λ <i>gcvT::lacZ</i> _{IHF-D}	<i>gcvR::Tn10</i>	302	376	234
1053 λ <i>gcvT::lacZ</i> _{IHF-G}	<i>gcvR::Tn10</i>	19	24	12
997 λ <i>gcvT::lacZ</i> _{H-P}	<i>lrp::Tn10</i>	24	35	8
997 λ <i>gcvT::lacZ</i> _{IHF-C}	<i>lrp::Tn10</i>	210	354	31
997 λ <i>gcvT::lacZ</i> _{IHF-D}	<i>lrp::Tn10</i>	174	339	22
997 λ <i>gcvT::lacZ</i> _{IHF-G}	<i>lrp::Tn10</i>	9	22	<1

The *gcvR* lysogens GS1053 IHF-C and IHF-D showed constitutively expressed levels of β -galactosidase activity under all growth conditions compared to the 852 λ *gcvT::lacZ*_{H-P} control lysogen (compare Tables 2 and 3). The levels were not significantly different in either lysogen, but were about threefold lower than the 1053 λ *gcvT::lacZ*_{H-P} control lysogen. The reduced levels of expression in the GS852 IHF-C and IHF-D lysogens compared to the GS1053 lysogens suggest that the role of GcvR as a negative regulator in *gcvT::lacZ* expression, which is dependent on GcvA, has not been eliminated.

The GS1053 IHF-G lysogen also showed an increase in β -galactosidase; its activity levels were not significantly different from those in the GS998 IHF-G lysogen (Table 3). Thus, if the low β -galactosidase levels observed in the GS852 IHF-G lysogen are due to GcvA-mediated super-repression, the repression is partially dependent on GcvR.

GcvA-mediated regulation in the I1A-insertion lysogens is IHF-dependent

If the glycine-mediated activation and inosine-mediated repression observed in the GS852 IHF-C and IHF-D lysogens are due to IHF binding and bending the *gcv* DNA, this activation and repression should not be observed in a mutant missing this protein. In addition, if the superrepression observed in the GS852 IHF-G lysogen is dependent on IHF binding and bending *gcv* DNA, this repression should be relieved in the absence of IHF. To test these hypotheses, the *ihfB::Cm^r* mutation was transduced into the control 852 λ *gcvT::lacZ*_{H-P} and the GS852 IHF-C, IHF-D, and IHF-G lysogens. The *ihfB::Cm^r* transductants were

designated 852*ihfB::Cm^r* λ *gcvT::lacZ*_{H-P}, etc., to indicate that they were constructed by transduction of pre-existing λ lysogens rather than by lysogenizing an IHF⁻ strain. This was necessary due to the inability to lysogenize an IHF⁻ strain with λ phage (Friedman, 1988). The lysogens were grown in GM medium with appropriate supplements, and β -galactosidase levels were measured. The control lysogen 852*ihfB::Cm^r* λ *gcvT::lacZ*_{H-P} showed about sixfold glycine-mediated activation and about fivefold inosine-mediated repression (Table 4). These results are similar to the results for the 852 λ *gcvT::lacZ*_{H-P} lysogen, although the presence of the *ihfB::Cm^r* mutation did result in a slight reduction in β -galactosidase levels (compare Tables 2 and 4). Both the GS852*ihfB::Cm^r* IHF-C and IHF-D lysogens showed about 1.5- to 2-fold glycine-mediated activation, although the induced β -galactosidase levels were 8.5- to 11-fold lower than the control lysogen (Table 4). In addition, both lysogens showed essentially non-repressible levels of β -galactosidase compared to the control lysogen (Table 4). These results are consistent with the hypothesis that an IHF-induced DNA bend is required for activation and repression in the GS852 IHF-C and IHF-D lysogens. The results are also consistent with a model where Lrp normally plays primarily a structural role, and GcvA is the *gcv*-specific regulatory protein. It should be emphasized, however, that there is an inability of GcvA and IHF to fully activate the *gcvT::lacZ* IHF-C and IHF-D fusions even in a *gcvR* background (Table 3). Because of the large number of Lrp-binding sites in the *gcv* control region (Stauffer & Stauffer, 1994), it was not possible to determine the overall degree of bending when all of the Lrp binding sites were occupied (Stauffer & Stauffer, 1998b). IHF bound to the phage λ I1A site

Table 4. Expression of *gcvT::lacZ* in the IHF lysogens is IHF-dependent

Lysogens were grown in GM medium plus the indicated supplements. All values were within 10% of the mean.

Lysogen	Relevant genotype	β -Galactosidase activity (Miller units)		
		None	Glycine	Inosine
852 <i>ihfB</i> ::Cm ^r λ <i>gcvT</i> :: <i>lacZ</i> _{IHF-P}	<i>purR6</i> ::Tn10	95	571	18
852 <i>ihfB</i> ::Cm ^r λ <i>gcvT</i> :: <i>lacZ</i> _{IHF-C}	<i>purR6</i> ::Tn10	36	52	26
852 <i>ihfB</i> ::Cm ^r λ <i>gcvT</i> :: <i>lacZ</i> _{IHF-D}	<i>purR6</i> ::Tn10	31	67	26
852 <i>ihfB</i> ::Cm ^r λ <i>gcvT</i> :: <i>lacZ</i> _{IHF-G}	<i>purR6</i> ::Tn10	19	23	11

bends the DNA about 140° (Xin & Feiss, 1993). Thus, it is possible that the inability of IHF to activate the *gcvT::lacZ* fusion fully is because the degree of the IHF-induced DNA bend is not precise enough to allow for all of the required protein–protein contacts necessary for activation and repression. However, it is also possible that Lrp plays an additional, more direct, role, interacting with one of the other regulatory proteins or with RNAP. If this is an Lrp-specific interaction, presumably this interaction cannot occur with IHF bound to *gcv* DNA. It has been shown that the RNAP α subunit is capable of contacting different factors independently (Murakami *et al.*, 1997), and that the σ subunit of RNAP interacts with cI protein (Kuldell & Hochschild, 1994). Thus, it is possible that both GcvA and Lrp interact directly with RNAP.

The GS852*ihfB*::Cm^r IHF-G lysogen showed β -galactosidase levels in GM, GM + glycine and GM + inosine that were elevated significantly compared to those found in the GS852 IHF-G lysogen (compare Tables 2 and 4). These levels were not significantly different from those observed in the GS998 and GS1053 IHF-G lysogens; this suggests that the superrepressed levels of β -galactosidase observed in the GS852 IHF-G lysogen require that IHF presumably binds and bends the DNA. Possibly the IHF-induced bend itself or, alternatively, a protein or DNA interaction that occurs with RNAP as a result of the bend destabilizes the transcriptional apparatus, reducing *gcvT::lacZ* expression. It has been proposed that a CRP-induced bend of DNA might facilitate the binding of upstream DNA to the backside of RNAP (Bracco *et al.*, 1989; Gartenberg & Crothers, 1991), and such an interaction could either destabilize or stabilize the transcription complex, decreasing or increasing transcription initiation.

Role of Lrp in *gcvT::lacZ* expression in the IHF-C, IHF-D and IHF-G mutants

Since all but the upstream 22 bp of the Lrp-binding region was replaced in the IHF lysogens, we predicted that Lrp would no longer be involved in regulation of the *gcvT::lacZ* fusion. Thus, λ phages carrying the IHF-

C, IHF-D and IHF-G insertions, as well as the control λ *gcvT*::*lacZ*_{IHF-P} phage, were used to lysogenize the *lrp* strain GS997, the lysogens were grown in GM medium with the appropriate supplements, and β -galactosidase levels were measured. As expected, the 997 λ *gcvT*::*lacZ*_{IHF-P} lysogen, with an intact Lrp-binding region, showed low levels of β -galactosidase that were essentially non-inducible by glycine or repressible by inosine, except for the expected PurR-mediated repression (Table 3). In contrast, the GS997 IHF-C and IHF-D lysogens showed about twofold higher levels of β -galactosidase in GM medium compared to the GS852 IHF-C and IHF-D lysogens (compare Table 2 and Table 3). These levels were induced about twofold in the presence of glycine, to about the same levels observed in the GS852 and GS1053 lysogens. In addition, there was about a sevenfold and eightfold inosine-mediated repression in the GS997 IHF-C and IHF-D lysogens, respectively (about twofold is PurR-mediated). Thus, Lrp has an effect on *gcvT::lacZ* expression in the IHF-C and IHF-D lysogens, but its effect is different from the normal Lrp role, where Lrp has been shown to be required for activation and repression of the operon (Stauffer & Stauffer, 1994). The *lrp* mutation also partially relieved the superrepression observed in the GS852 IHF-G lysogen (Table 3).

Leucine supplementation of the growth medium modifies the action of Lrp at some promoters (Calvo & Matthews, 1994; Newman *et al.*, 1996), but has no effect on *gcv* expression (Lin *et al.*, 1992). We tested whether leucine supplementation alters the Lrp effect on *gcvT::lacZ* expression in the 852 λ *gcvT*::*lacZ*_{IHF-P} and 852 λ *gcvT*::*lacZ*_{IHF-C} lysogens. There was no significant difference in either lysogen grown in GM or GM + glycine and either with or without a leucine supplement (data not shown).

Lrp-binding analysis

We used a gel mobility-shift assay to determine whether Lrp could still bind to the *gcv* control region carrying the insertion sequences, and this binding could be responsible for the negative effect of Lrp on *gcvT::lacZ* regulation. A ³²P-labelled DNA fragment carrying the IHF-C region was shifted to two positions of slower

Fig. 2. Gel mobility-shift assay for Lrp. (a) A 759 bp 32 P-labelled *EcoRI*-*Bam*HI fragment carrying the *gcv* control region from pGS452 (IHF-C) was incubated with dilutions of Lrp, and the samples were run on a 5% polyacrylamide gel. Lanes 1–7 are 5, 10, 21, 42, 83, 166 and 332 nM Lrp dimer, respectively; lane 8, no Lrp. (b) A 373 bp 32 P-labelled *Bam*HI fragment from pGS466 (wild-type Lrp-binding region) was incubated with dilutions of Lrp, and the samples were run on a 5% polyacrylamide gel. Lanes 1–7 are 0.6, 1.25, 2.5, 5, 10, 21, and 42 nM Lrp dimer, respectively; lane 8, no Lrp. Arrows indicate the positions of unbound fragments.

mobility as the Lrp-dimer concentration was increased from 5 to 10 nM (Fig. 2a, lanes 1 and 2). At 21 nM dimer the amount of unbound DNA decreased and the amount of bound DNA increased, and at 42 nM dimer all of the labelled fragment was shifted upwards (Fig. 2a). Because of the large size of the fragment used as a probe (769 bp), the bound DNA barely migrated into the gel, and it was difficult to determine whether the band containing the bound DNA was actually a single or multiple bands. We quantified the amount of unbound fragment at each Lrp concentration. The results suggest that more than 50% of the labelled probe was shifted at an Lrp-dimer concentration of about 24 nM. As a control, we also used a 339 bp 32 P-labelled probe carrying the wild-type Lrp-binding region. As the Lrp-dimer concentration was increased from about 0.6 to 10 nM dimer, the DNA fragment was shifted to at least six positions of slower mobility, and at 20 nM, all of the DNA was shifted to the most slowly migrating species (Fig. 2b). More than 50% of the labelled DNA fragment was shifted at an Lrp-dimer concentration of about 2 nM. This is similar to the affinity determined previously for Lrp binding to

the wild-type *gcv* control region (50% binding at an Lrp dimer concentration of about 2.2 nM) (Stauffer & Stauffer, 1994). Thus, although the IHF-C DNA fragment binds Lrp, the affinity is reduced more than 10-fold compared to a fragment carrying the wild-type Lrp binding region. This is about fourfold higher than the estimated physiologically relevant level of Lrp in the cell (5.5 nM) (Borst *et al.*, 1996). Thus, it is likely that the Lrp-responsiveness of *gcvT::lacZ* expression in the IHF lysogens is indirect rather than due to weak binding of Lrp to *gcv* DNA.

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