Characterization of the gcv Control Region from Escherichia coli

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We constructed a set of deletions upstream of the gcv promoter and analyzed the effects of the deletions on expression of a gcvT-lacZ gene fusion. A deletion that ends at position -313 upstream of the transcription initiation site (+1) results in reduced levels of gcvT-lacZ expression, but the fusion is still inducible by glycine and repressible by purines. A deletion that ends at position -169 results in loss of both GcvA- and Lrp-mediated activation of the gcvT-lacZ fusion. The endpoints of Δ-313 and Δ-169 also define a site that down-regulates gcvT-lacZ expression two- to threefold. A deletion that ends at position -89 upstream from the transcription initiation site still shows PurR-mediated repression, suggesting that PurR-mediated repression is not by direct interference with the GcvA- and Lrp-mediated regulatory mechanism(s). Gel mobility shift assays and DNase I footprinting showed that Lrp protein binds to multiple sites upstream of the gcv promoter, from about bp -92 to bp -229. The results suggest that the gcv regulatory region is complex, with numerous cis-acting sites that are required for normal gcv expression.

Serine hydroxymethyltransferase catalyzes the conversion of serine to glycine and 5,10-methylenetetrahydrofolate and is the major source of one-carbon units for cell metabolism (14). The glycine cleavage (GCV) enzyme system catalyzes the oxidative cleavage of glycine to carbon dioxide, ammonia, and 5,10-methylenetetrahydrofolate and provides a secondary pathway for one-carbon biosynthesis (8, 12, 20). The GCV enzymes, however, have only about 1/10 the activity of serine hydroxymethyltransferase (8). The low activity of the GCV enzyme system is believed to ensure that sufficient levels of glycine are maintained for other pathways that require this amino acid (purine synthesis and protein synthesis, etc.).

The GCV enzyme system is inducible by glycine, and at least two proteins, GcvA (31) and Lrp (10), are required for gcv expression. GcvA activates gcv expression in the presence of exogenous glycine and represses gcv in the presence of exogenous purines without glycine (30). Lrp activates gcv but is insensitive to the coregulator leucine (7, 10). In addition, PurR, a repressor protein involved in negatively regulating purine nucleotide synthesis (9, 18, 19), also is involved in negative regulation of the GCV enzyme system (30). Whether these proteins act through a common mechanism or function independently is unknown.

Previously, genetic and biochemical methods were used to define the promoter region for the gcv operon (15, 24). The PurR-binding site was shown to overlap the gcv promoter (from about nucleotide -3 to +17 relative to the transcription initiation site) (30). Whether GcvA and Lrp bind directly to the gcv control region and where these proteins bind are unknown. To understand the DNA sequence elements required for gcv expression, we have extended the genetic and biochemical analysis of the gcv regulatory region. Our results suggest that the gcv regulatory region is complex, having several cis-acting regulatory sites required for gcv expression. The results confirm that both GcvA and Lrp are required for expression of gcv and that sequences greater than 313 bp upstream of +1 are required for normal gcv expression. In addition, the results show that Lrp binds to multiple sites upstream of the gcv promoter, suggesting a direct role for Lrp in gcv expression.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. The bacterial strains used in this study are listed in Table 1. Phage Agt2 (16) was obtained from R. Davis, and XcI857c17 (23) was obtained from M. Feiss. Plasmid vectors pBR322 (2) and pMC1403 (3) have been described previously. Plasmid pGS146 carries the gcv operon (25).

Media. Luria broth and Luria agar were used as rich media (13). The minimal medium was the minimal salts of Vogel and Bonner (27) supplemented with 0.4% glucose (GM medium). GM medium was always supplemented with phenylalanine and vitamin B1, since most strains carry the pheA905 thi mutations. Other supplements were added at the following concentrations (in micrograms per milliliter): amino acids, 50 (glycine was added at 300); vitamins, 1; ampicillin, 150; chloramphenicol, 20; tetracycline, 10; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 40.

The λ lysogens carry the cI857 mutation, resulting in a temperature-sensitive repressor, and were grown at 30°C. Lysogens were tested for a single copy of the bacteriophage by infection with λc190c17 (23).

Enzyme assays. β-Galactosidase activity was assayed as described by Miller (13) by using the chloroform-sodium

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**TABLE 1. Strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
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<tbody>
<tr>
<td>CV1008*</td>
<td>F' ara thi Δ(lac-pro) livH::MudI1734 lrp::Tn10</td>
</tr>
<tr>
<td>GS162</td>
<td>pheA905 araD129 ΔacU169 rpsL thi GS852</td>
</tr>
<tr>
<td>GS997</td>
<td>pheA905 araD129 ΔacU169 rpsL thi::Tn10</td>
</tr>
<tr>
<td>GS998</td>
<td>pheA905 araD129 ΔacU169 rpsL thi gcvAl</td>
</tr>
<tr>
<td>GS1003</td>
<td>pheA905 araD129 ΔacU169 rpsL thi::Tn10 gcvAl</td>
</tr>
</tbody>
</table>

* From J. Calvo.

* Originally designated ihb::Tn10; the ihb allele has been renamed lrp, which is used throughout the text.
dodecyl sulfate lysis procedure. All results are averages of two or more experiments in which each sample was determined in triplicate.

**General procedures.** Procedures for plasmid DNA isolation, restriction enzyme digestion, and ligation, etc., were as described by Sambrook et al. (21). DNA sequencing was done by the method of Maxam and Gilbert (11). Phage DNA was isolated by using a modification (32) of the method of Benson and Taylor (1).

**Deletion construction.** Plasmid pGS239 carries a gcvT-lacZ gene fusion beginning at the KpnI site (position -466 relative to the gcv transcription initiation site) (Fig. 1) and was described previously (24). Other plasmids with deletions entering the gcv control region from the 5' flanking region were constructed as follows. EcoRI linkers were ligated to the NruI (position -89), SspI (position -169), and BsaBI (position -313) sites upstream of the gcv promoter in plasmid pGS239 (made blunt, if necessary, with T4 DNA polymerase) (Fig. 2). The DNA was digested with EcoRI and BamHI, and the EcoRI-BamHI DNA fragments carrying the gcv promoter region and the beginning of the gcvT structural gene were isolated and ligated into the EcoRI and BamHI sites of the lac fusion vector pMC1403, generating plasmids pGS256(Δ-89), pGS257(Δ-169), and pGS258(Δ-313). This created an in-frame fusion of the gcvT structural gene to the lacZ gene in each plasmid. Plasmid pGS259(Δ-1167) was constructed by converting an EcoRV site upstream of the gcv promoter in plasmid pGS146 to an EcoRI site. The EcoRI-BsaBI fragment was isolated from this intermediate plasmid and used to replace the EcoRI-BsaBI fragment in plasmid pGS239, retaining the in-frame fusion of the gcvT structural gene to lacZ in plasmid pGS239. An approximately 7- to 8-kbp EcoRI DNA fragment carrying each gcvT-lacZ fusion along with the lacY and lacZ genes was isolated from each plasmid and cloned into the EcoRI site of phage λgt2 by the method described previously (26), generating phages λTlac(Δ-89), λTlac(Δ-169),

![Fig. 1. Schematic of construction of deletion plasmids. A segment of the 7.12-kbp SalI-BamHI DNA fragment in plasmid pGS146 that carries the region 5' to the gcv control region and the beginning of the first structural gene (gcvT) is shown. Only relevant restriction enzyme recognition sites are indicated. +1 is the transcription initiation site for gcv determined previously (24). The fusion site of gcvT to lacZ in plasmid pGS239 is at the SmaI site (24). The KpnI site in pGS146 was converted to an EcoRI site in the construction of pGS259. The construction of other deletion plasmids is described in Materials and Methods. The deletion number of each plasmid indicates the 5' endpoint of the deletion.](http://jb.asm.org/content/104/11/6160/F1)

![Fig. 2. DNA sequence of the gcv control region. The transcription start site (+1), the −10 and −35 promoter sequence elements, and the PurR-binding site were determined previously (24, 30). The restriction sites used to generate the deletion plasmids (Fig. 1) are indicated above the sequence. The region protected by Lrp from DNase I attack (Fig. 4) is indicated by a bar above the sequence.](http://jb.asm.org/content/104/11/6160/F2)
αTlac(Δ−313), αTlac(Δ−466) and λTlac(Δ−1167). The Δ number in each case represents the 5' endpoint of the gcv sequence joined with the EcoRI linker.

**Gel mobility shift assay.** The gel mobility shift assay was based on the methods of Fried and Crothers (5) and Garner and Revzin (6). A double-stranded 769-bp EcoRI-BamHI fragment carrying the gcv control region from plasmid pGS239 (24) was 32P labeled at the EcoRI end by using the large fragment of DNA polymerase I (21). Less than 0.2 ng of the labeled fragment was added to 20-μl reaction mixtures containing 1× DNA-binding buffer (10 mM Tris hydrochloride [pH 7.5], 50 mM KCl, 0.5 mM EDTA, 5% glycerol, 1 mM dithiothreitol) plus 125 μg of bovine serum albumin (BSA) per ml. The reaction mixtures were preincubated at 37°C for 5 min, 2 μl of purified Lrp protein was added to the reaction mixtures as indicated in Fig. 3, and incubation was continued for 15 min. One microliter of loading dye (0.1% xylene cyanol and 50% glycerol in H2O) was added to each reaction mixture, and the samples were loaded immediately onto a 5% polyacrylamide-3% glycin gel and run at 12 V/cm for 3.5 h. Gels were transferred to 3MM Whatman paper, dried, and autoradiographed.

**DNase I protection assay.** The DNase I protection assay was based on the method of Schmitz and Galas (22). The 769-bp 32P-labeled EcoRI-BamHI fragment used in the gel mobility shift assay was used in the DNase I footprint assay. Less than 2 ng of labeled DNA was added to 25-μl reaction mixtures containing 1× DNA-binding buffer plus 125 μg of BSA per ml. The reaction mixtures were preincubated at 37°C for 5 min, 2 μl of Lrp protein was added to the reaction mixtures as indicated in Fig. 4, and incubation was continued for an additional 15 min. A 1.25-μl portion of a DNase I solution (2.5 μg/ml in 20 mM sodium acetate [pH 7] plus 32 mM CaCl2) was added, and incubation was continued at 37°C for 30 s. Reactions were stopped by adding 5 μl of stop solution (3 M ammonium acetate, 15 μg of sonicated calf thymus DNA per ml, 0.25 M EDTA). Samples were precipitated, resuspended in loading buffer, and loaded onto a 5% polyacrylamide-urea sequencing gel alongside the A+G and C+T sequencing reactions (11) of the same DNA fragment.

**RESULTS**

**Effects of deletion mutations on gcvT-lacZ expression.** Three proteins are known to be involved in regulation of the gcv operon. The GcvA (31) and Lrp (10) proteins are required for expression of the gcv operon, and the GcvA and PurR proteins (30) are involved in negative regulation of the gcv operon. The binding site for PurR was defined previously (30) and occurs from about nucleotide −3 to +17 relative to the transcription initiation site (+1). The binding sites for GcvA and Lrp have not been defined. Starting with a gcvT-lacZ gene fusion, we constructed a series of 5' deletions to define the upstream limits of the gcv control region (see Materials and Methods) (Fig. 1). λgt2 phage carrying the deletions was used to lysogenize strain GS162, the lysogens were grown in GM medium with appropriate supplements, and β-galactosidase levels were measured. As reported previously (24, 30, 31), glycin induced gcvT-lacZ expression in lysogen GS162Tlac(Δ−466) more than sixfold (Table 2). In the presence of both glycine and inosine, the induced gcvT-lacZ expression was reduced about twofold compared with that in the presence of glycine alone as a result of PurR-mediated repression. In the absence of glycine, inosine repressed expression more than ninefold; this was shown previously to be a result of a combined repression by PurR and GcvA. Lysogen GS162Tlac(Δ−1167) has an additional 701 bp of upstream sequence compared with lysogen GS162Tlac(Δ−466). However, gcvT-lacZ expression is essentially the same in both lysogens (Table 2), suggesting that sequences further upstream than position −466 are not necessary for normal gcv expression.

In lysogen GS162Tlac(Δ−313), gcvT-lacZ expression was reduced compared with that of lysogen GS162Tlac(Δ−466). However, glycine still induced gcvT-lacZ expression, and inosine still repressed gcvT-lacZ expression in both the presence and absence of glycine (Table 2). In lysogens GS162Tlac(Δ−169) and GS162Tlac(Δ−89), induction by glycine was abolished, and the twofold PurR-mediated repression remained. To verify that this residual repression is PurR mediated, the purR mutant strain GS852 was lysogenized with λTlac(Δ−466), λTlac(Δ−313), and λTlac(Δ−169). In lysogen GS852Tlac(Δ−466), the twofold repression observed in the presence of both glycine and inosine compared with that in the presence of glycine alone was no longer seen (Table 2). However, as reported previously (30), inosine alone still repressed gcvT-lacZ expression via the GcvA protein. In lysogen GS852Tlac(Δ−313), although gcvT-lacZ expression was reduced compared with that of GS852Tlac(Δ−466), the PurR-mediated repression was no longer seen in the presence of both glycine and inosine, but inosine alone still repressed gcvT-lacZ expression. In lysogen GS852Tlac(Δ−169), inosine supplementation no longer repressed gcvT-lacZ expression.

**Effects of the deletion mutations in gcvA and lrp mutants.** To determine the effects of the deletions in a strain lacking a GcvA activator protein, we lysogenized the gcvA mutant strain GS998 with phase λTlac(Δ−466), λTlac(Δ−313), and λTlac(Δ−169). The gcvA mutant lysogen GS998Tlac(Δ−466) was noninducible by glycine and nonrepressible by inosine, except for the twofold PurR-mediated repression (Table 3). In lysogen GS998Tlac(Δ−313), again gcvT-lacZ expression was noninducible by glycine and nonrepressible by inosine, except for the twofold PurR-mediated repression. However, β-galactosidase levels were reduced about three- to fourfold under all growth conditions compared with those of GS998Tlac(Δ−466). A deletion ending at position −169 increased the basal level of expression about twofold compared with that of GS998Tlac(Δ−313), suggesting that a negative-acting site was removed. Again, gcvT-lacZ was noninducible by glycine and nonrepressible by inosine, except for the twofold PurR-mediated repression.

To determine the effects of the deletions in a strain lacking the Lrp protein, we lysogenized the lrp mutant strain GS997 with phase λTlac(Δ−466), λTlac(Δ−313), and λTlac(Δ−169). In lysogen GS997Tlac(Δ−466), grown in GM medium, gcvT-lacZ expression was about threefold lower than it was in the

**TABLE 2. Effects of deletions upstream of the gcv transcription initiation site on gcvT-lacZ expression**

<table>
<thead>
<tr>
<th>Lysogena</th>
<th>Relevant genotype</th>
<th>β-Galactosidase activityb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
<td>Glycine</td>
</tr>
<tr>
<td>GS162Tlac(Δ−1167) Wild type</td>
<td>136</td>
<td>801</td>
</tr>
<tr>
<td>GS162Tlac(Δ−466) Wild type</td>
<td>123</td>
<td>750</td>
</tr>
<tr>
<td>GS162Tlac(Δ−313) Wild type</td>
<td>41</td>
<td>181</td>
</tr>
<tr>
<td>GS162Tlac(Δ−169) Wild type</td>
<td>43</td>
<td>41</td>
</tr>
<tr>
<td>GS162Tlac(Δ−89) Wild type</td>
<td>55</td>
<td>36</td>
</tr>
<tr>
<td>GS852Tlac(Δ−466) purR::Tn10</td>
<td>25</td>
<td>782</td>
</tr>
<tr>
<td>GS852Tlac(Δ−313) purR::Tn10</td>
<td>53</td>
<td>220</td>
</tr>
<tr>
<td>GS852Tlac(Δ−169) purR::Tn10</td>
<td>50</td>
<td>47</td>
</tr>
</tbody>
</table>

*a The growth medium was GM medium plus the indicated supplements.

*b Units of activity are Miller units (13). Values varied by less than 15%.

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gcvA lysogen GS998Tlac(Δ−466) and was essentially noninducible by glycine (Table 3). However, when cells were grown in the presence of both glycine and inosine, expression was reduced about twofold compared with cells grown in glycine alone, and in the presence of inosine alone, expression was reduced about threefold (Table 3).

In lysogen GS997Tlac(Δ−313), gcvT-lacZ expression was reduced under all conditions compared with that of lysogen GS997Tlac(Δ−466). Although gcvT-lacZ expression was noninducible by glycine, repression by inosine was still observed, and this repression was more pronounced in the absence of glycine. A deletion ending at position −169 increased gcvT-lacZ expression about threefold compared with that of GS997 λTlac(Δ−313), supporting the above results that a negative-acting site lies between positions −169 and −313.

To determine whether the repression seen in GS997λTlac(Δ−466) by inosine alone was PurR or GcvA mediated, we also lysogenized GS997 with phage λTlac(Δ−466 +8C+10A) (+8C+10A indicates that nucleotide +8 is changed to C and that nucleotide +10 is changed to A). This phage carries a 2-bp change in the PurR-binding site and prevents PurR-mediated repression of the gcvT-lacZ fusion (30). In addition, the 2-bp change results in a twofold promoter-down phenotype (30). As shown in Table 3, the twofold PurR-mediated repression of gcvT-lacZ is not seen when inosine is added to the glycine-containing culture. However, inosine alone still resulted in low but significant repression.

We also tested the effects of the deletions in the gcvA lp double mutant GS1003. For the most part, β-galactosidase levels were essentially the same as in the gs997 lp lysogens, with one notable exception. Lysogen GS1003 λTlac(Δ−466 +8C+10A), grown in the presence of inosine, did not show any significant purine-mediated repression (Table 3).

**Lrp-binding analysis.** We used a gel mobility shift assay to determine whether the Lrp protein regulates gcv directly by binding to the gcv control region (see Materials and Methods). A labeled DNA fragment carrying the gcv control region was shifted to at least four positions of slower mobility while the Lrp dimer concentration was increased from 0.27 to 69 nM (Fig. 3). As the concentration of Lrp was increased, band A remained in small amounts, while band B increased to become the dominant species at 2 nM Lrp. Band C may be an intermediate complex and only appears in small amounts. Although not well resolved in Fig. 3, band C is a discrete band in other gels (data not shown). At higher concentrations of Lrp (greater than 4.3 nM), all of the complexes were shifted to band D. Concentrations of Lrp greater than 17 nM began to shift a control fragment isolated from within the amp gene of pHJ322 (data not shown). Thus, it is likely that bands A through D are the result of specific binding of Lrp to the labeled gcv fragment. Because of the large size of the DNA probe (769 bp) and the large number of Lrp dimers bound, it is difficult to determine if band D consists of a single species or multiple bands.

**DNase I footprint analysis.** The gel mobility shift assay suggested that Lrp binds to multiple sites in the gcv control region. To determine where the Lrp protein binds in the gcv control region, we used a DNase I footprint assay (see Materials and Methods). As the Lrp dimer concentration was increased from 0.22 to 27.6 nM, Lrp bound to and protected sequences spanning about a 137-bp region from position −92 to position −229 (Fig. 4, bars to the right of the gels). Within the protected region are sites that are either unphosphorylated or that show enhanced cleavage. There are additional sites outside of the protected region that also show enhanced cleavage. At higher Lrp concentrations (Fig. 4, lanes 2 and 3), Lrp appears to bind and protect nonspecifically, consistent with results from the gel mobility shift assay. Because DNase I did not cleave randomly, it is difficult to define precisely the boundaries of each target site or the precise boundaries of the Lrp-protected region.

**DISCUSSION**

Both the Lrp and GcvA proteins are necessary for expression of the gcv operon (10, 31). Deletions extending into the gcv control region from the 5′ side to position −466 show normal induction by glycine and repression by inosine (Table 2). A deletion ending at position −313 results in reduced expression of gcv but still allows significant induction by glycine and repression by inosine. However, the observed repression by inosine alone is not reduced as much as the other conditions. Although the mechanism for the reduced expression in Δ−313 is unknown, the deletion has not altered equally the abilities to activate by glycine and to repress by inosine. A deletion ending at position −169 abolishes both GcvA- and Lrp-mediated activation as well as GcvA-mediated purine repression (Table 2). These results suggest that DNA-binding sites for one or both of these proteins is greater than 169 bp.
upstream of the transcription initiation site. The results also suggest that Δ−313 could disrupt a sequence necessary for maintaining high levels of gcv expression.

In the gcvA mutant lysogen GS998, deletion of the sequence between −169 and −313 resulted in a twofold increase in gcvT-lacZ expression under all growth conditions (Table 3). In

the lrp or lrp gcvA mutant lysogens GS997 and GS1003, deletion of the sequence between −169 and −313 resulted in about a threefold increase in gcvT-lacZ expression under all growth conditions. In these mutant lysogens, the increase in gcvT-lacZ expression is raised only to the level found in the wild-type strain GS162ATlac(Δ−169) (Table 2). These results suggest that there is an additional site that reduces gcv expression but only in the absence of the GcvA or Lrp protein.

One possibility is that the DNA sequence itself is important for gcv expression, possibly by bending DNA, or that this region could define a target site for an additional regulatory protein. Studies to distinguish between these possibilities are in progress.

In the lrp lysogen GS997ATlac(Δ−466+8C+10A), glycine fails to induce gcvT-lacZ expression. However, inosine still resulted in about a twofold repression of gcvT-lacZ expression (Table 3). Since the PurR-binding site was inactivated by the 2-bp change (31) and Lrp was inactivated by the Tn10 insertion, the results suggest that GcvA could be responsible for the purine-mediated repression. This conclusion is supported by results with lysogen GS1003ATlac(Δ−466+8C+10A), where both Lrp and GcvA are nonfunctional and no significant purine-mediated repression is observed. It should be noted, however, that the combination of the lrp and gcvA mutations and the promoter-down effect of the +8C+10A base pair changes significantly reduce β-galactosidase levels. Nevertheless, the results suggest that GcvA could be responsible for the purine-mediated repression in lysogen GS997ATlac(Δ−466+8C+10A) and that GcvA might be able to bind to DNA in the absence of Lrp to repress gcvT-lacZ expression. Additional studies will be necessary to confirm or refute this hypothesis.

Lrp binds the ihH operon in two steps (28). At low concentrations, Lrp binds to two sites which are more than 200 bp upstream of the transcription start site in a cooperative step. At higher concentrations, Lrp dimers bind cooperatively to four additional sites near the transcription start site, spanning about a 200-bp region. Binding of Lrp dimers to all six sites is required for activation of the ihH promoter. It was proposed that the multiple binding sites result in the formation of a nuclear protein complex such that a particular proximal site properly positions Lrp so that it is capable of interacting with RNA polymerase. Our results with the gel shift assay suggest that multiple Lrp-binding sites occur in the gcv control region since several slower-migrating bands are seen as the Lrp concentration is increased (Fig. 3). Results from a DNase I footprint assay showed that Lrp binds to multiple sites in the gcv control region, spanning a region from about −92 to −229 relative to the transcription initiation site. However, because of the nonrandom cleavage of the DNA fragment by DNase I, the precise boundaries for Lrp binding and the number of binding sites could not be determined. In addition, there are sites within this region that are unprotected and sites both within and outside of this region that show enhanced cleavage in the presence of Lrp. Since Lrp binds DNA (28), if Lrp binding in the gcv control region bends the DNA, certain nucleotides may be more susceptible to DNase I attack. The results suggest that Lrp might also form a nuclear protein complex to control regulation of the gcv operon. This picture is further complicated, however, since the GcvA protein also appears to bind multiple sites upstream of the gcv promoter (29).

Lrp-mediated activation of gcv is independent of leucine (7, 10). Ernsting et al. (4) proposed a model for Lrp regulation, suggesting that operons insensitive to leucine would be expected if the effective intracellular concentration of Lrp was high relative to the affinity of Lrp for the binding sites for
transcription of the target operon. It was estimated that the effective intracellular Lrp concentration is approximately 5.5 nM in minimal medium (4). Because of the multiple binding sites for Lrp in the gcv control region, we did not calculate dissociation constants for Lrp binding. However, results from gel mobility shift assays show that more than 50% of the labeled target fragment is shifted at an Lrp concentration of 2.2 nM (Fig. 3), suggesting that at least one of the binding sites has a high affinity for Lrp, and provide a possible explanation for the insensitivity of gcv to leucine. In a preliminary gel mobility shift assay, leucine (10 mM) reduced the affinity of Lrp for the gcv operon (data not shown). We are preparing overlapping DNA fragments with individual and multiple binding sites to determine their affinity for Lrp, whether Lrp binds cooperatively, and the effect of leucine on binding. These experiments, coupled with a genetic analysis, should define the sites necessary for Lrp-mediated activation of gcv.

Wang and Calvo (28) proposed an Lrp consensus binding sequence of 5'-AGAATTTATTCAT by alignment of the six Lrp-binding sites for ilvHJ. This contains in part a consensus proposed by Rex et al. (17) of 5'-TTATTCATNaAT (lowercase letters indicate nucleotides that are not conserved 100% in all known Lrp-binding sites) made by aligning sequences in the 5' upstream regions of genes that belong to the leucine regulon. Although partial matches can be identified in the gcv control region protected by Lrp from DNase I digestion, most sites show multiple changes from the proposed consensus sequences. The precise sequence recognized by Lrp in the gcv control region will require more extensive genetic and biochemical experiments.

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REFERENCES
