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Characterization of the Interaction of the *glp* Repressor of *Escherichia coli* K-12 with Single and Tandem *glp* Operator Variants

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The *glp* operons of *Escherichia coli* are negatively controlled by the *glp* repressor. Comparison of the repressor-binding affinities for consensus and altered consensus operators in vivo showed that all base substitutions at positions 3, 4, 5, and 8 from the center of the palindromic operator caused a striking decrease in repressor binding. Substitutions at other positions had a severe to no effect on repressor binding, depending on the base substitution. The results obtained indicate that the repressor binds with highest affinity to operators with the half-site WATKYTCGWW, where W is A or T, K is G or T, and Y is C or T. Strong cooperative binding of the repressor to tandem operators was demonstrated in vivo. Cooperativity was maximal when two 20-bp operators were directly repeated or when 2 bp separated the two operators. Cooperativity decreased with the deletion of 2 bp or the addition of 4 bp between the individual operators. Cooperativity was eliminated with a 6-bp insertion between the operators.

The proteins that catalyze the steps required for the utilization of glycerol, glycerol-3-phosphate (glycerol-P), and glycerophosphodiesters are encoded by the *glp* regulon of *Escherichia coli* (14). The *glp* regulon is composed of five operons located at three different regions on the linkage map of *E. coli*. Transcription of the *glp* operons is negatively regulated by the *glp* repressor, a tetrameric protein encoded by the *glpR* gene (13, 17). Negative control is mediated by binding of the *glp* repressor to its operator sites within the *glp* operons. The affinity of the repressor for its operators is decreased in the presence of glycerol-P, the inducer for the regulon (14). Thirteen operators have been identified in the *glp* operons by using DNase I footprinting (12, 22, 23). The operators match more or less well the consensus operator 5′-WATGTTCGWT · AWCGAACA TW-3′ (W is A or T, and the dot indicates the center of symmetry) (22). Tandemly repeated repressor-binding sites are present in the control regions of the *glpACB*, *glpD*, and *glpFKX* operons (12, 20, 22, 23). A single operator overlaps the CRP site of the *glpTQ* operon (12). Repressor-mediated DNA looping is involved in control of the divergently transcribed *glpTQ-glpACB* operons (12). The *glp* operons exhibit differential sensitivity to the repressor (14, 22). Differential control may be mediated by differences in the degree of similarity of the operators to the consensus sequence, the presence or absence of cooperative binding to multiple operators, differences in the positions of the operators with respect to promoters elements, or combinations of the above factors (5, 22).

The present work was performed in order to define the operator sequence specificity for repressor binding. In addition, variously spaced tandem operators were constructed in order to assess the potential role of cooperative binding of the repressor to tandem operators for control of *glp* gene expression.

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MATERIALS AND METHODS

Bacterial strains and media. The bacterial strains utilized or constructed are shown in Table 1. Preparation of phage lysates and transductions with P1*vir* were carried out as described previously (18). For minimal media, the A and B salts described by Clark and Maaløe (4) were supplemented with 2 μg of thiamine per ml and 0.2 to 0.4% of the various carbon sources. Media contained 20 mM sodium citrate for the selection or scoring of transductants. Transformation of competent cells was carried out as described previously (18), with selection on LB plates (15) containing 100 μg of ampicillin per ml and 40 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml where appropriate.

Preparation of operator DNA. Oligonucleotides with the consensus or variant *glp* operator sequences were synthesized on an Applied Biosystems 381A DNA synthesizer and purified on oligonucleotide purification cartridges as recommended by the manufacturer (Applied Biosystems). In some cases, mixtures of phosphoramidite bases were coupled to generate multiple base substitutions at the targeted position. Operator duplexes were prepared with *Bam*HI and *Pst*I sites present on either end to facilitate cloning. An *Eco*RV site was present at the center of symmetry for the identification of recombinant plasmids containing operator DNA, for recombination of operator half-sites from different plasmids, and for insertion of an additional duplex to generate plasmids with tandemly repeated operators. The sequences of the oligonucleotides synthesized are shown in Table 2.

Cloning of operator DNA. Plasmid vector pGEM3Z (Promega) was digested with *Bam*HI and *Pst*I and purified following electrophoresis on low-gelling-temperature agarose. Equimolar amounts of the oligonucleotides to be cloned were mixed in 10 mM Tris-HCl (pH 8)–50 mM NaCl–1 mM EDTA and heated to 85°C. The annealing mixture was allowed to cool to room temperature for several hours. A 40-fold molar excess of annealed oligonucleotides was mixed and ligated (3 h) to 150 ng of cleaved vector DNA in 0.04 ml. An aliquot of the ligation mixture was used for transformation of strain DH5αF′.

TABLE 1. Strains of *E. coli* K-12

Strain	Genotype	Source or derivation
DH5αF′	F′ φ80d*lacZΔM15 recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1* Δ(*lacZ-argF*)*U169*	Bethesda Research Laboratories
ECL89	HfrC *glpR12* (*glpR*ⁿ) *phoA8 fhuA22 ompF627 fadL701 relA1 pit-10 spoT1*	E. C. C. Lin (6)
MC4100	F^- *araD139* Δ(*argF-lac*)*U169 rpsL150 deoC1 relA1 rbsR ptsF25 flbB5301*	M. J. Casadaban (3)
TS100	MC4100 *glpR2*	T. J. Silhavy (19)
SH305	MC4100 Δ*glpD102 recA1 srl*::Tn*10*	H. Schweizer (17)
TST3	MC4100 *malT*::Tn*10*	T. J. Silhavy (18)
TL681	MC4100 φ(*glpA101-lacZ*) λp1(209) Δ*glpD102 sdh-9*	T. J. Larson (7)
GD4	MC4100 *glpR2* φ(*glpD-lacZ*)*hyb* λp1(209) *zih-730*::Tn*10*	G. Sweet
GD6	TS100 φ(*glpD-lacZ*)*hyb* λp1(209)	P1(GD4)→TS100 (Lac^+ selection)
TJS1	ECL89 *malT*::Tn*10*	P1(TST3)→ECL89 (Tet^r selection)
TJS51	GD6 *glpR12 malT*::Tn*10*	P1(TJS1)→GD6 (Tet^r λimm selection)
TJS52	TJS51 *malT*$^+$	P1(TL681)→TJS51 (Mal^+ selection)
WO331	TJS52 *recA1 srl*::Tn*10*	P1(SH305)→TJS52 (Tet^r selection)

The presence of the desired operator insert was verified phenotypically (white colonies on LB supplemented with X-Gal), by restriction analysis, and by nucleotide sequence analysis.

The plasmid with the *glp* consensus operator served as the vector for construction of a series of plasmids with variously spaced tandemly repeated operators. This series was constructed by ligation of annealed self-complementary oligonucleotides (Table 2) into the *Eco*RV site present at the center of operator symmetry. The structures of the resulting plasmids were verified by restriction analysis and nucleotide sequence analysis.

TABLE 2. Oligonucleotides used for creation of *glp* operators

Operator	Nucleotide sequence[a]
glp consensus	GATCCTATGTTCGATATCGAACATACTGCA
	GATACAAGCTATAGCTTGTATG
7,7′	GATCCTAT(AC)TTCGATATCGAA(TG)ATACTGCA
	GATA(TG)AAGCTATAGCTT(AC)TATG
4,4′ A,T (*deo* operator)	GATCCTATGTTAGATATCTAACATACTGCA
	GATACAATCTATAGATTGTATG
1	GATCCTATGTTCGA(ACG)ATCGAACATACTGCA
	GATACAAGCT(TGC)TAGCTTGTATG
2	GATCCTATGTTCG(TCG)TATCGAACATACTGCA
	GATACAAGC(AGC)ATAGCTTGTATG
3	GATCCTATGTTC(ATC)ATATCGAACATACTGCA
	GATACAAG(TAG)TATAGCTTGTATG
4	GATCCTATGTT(ATG)GATATCGAACATACTGCA
	GATACAA(TAC)CTATAGCTTGTATG
5	GATCCTATGT(ACG)CGATATCGAACATACTGCA
	GATACA(TGC)GCTATAGCTTGTATG
6	GATCCTATG(ACG)TCGATATCGAACATACTGCA
	GATAC(TGC)AGCTATAGCTTGTATG
7	GATCCTAT(ATC)TTCGATATCGAACATACTGCA
	GATA(TAG)AAGCTATAGCTTGTATG
8	GATCCTA(ACG)GTTCGATATCGAACATACTGCA
	GAT(TGC)CAAGCTATAGCTTGTATG
9	GATCCT(TCG)TGTTCGATATCGAACATACTGCA
	GA(AGC)ACAAGCTATAGCTTGTATG
glp tandem	ATCGAACATATATGTTCGAT
deo tandem	ATCTAACATATATGTTAGAT
glp tandem (−2)	ATCGAACATATGTTCGAT
glp tandem (+2)	ATCGAACATATATATGTTCGAT
glp tandem (+4)	ATCGAACATATCGATATGTTCGAT
glp tandem (+6)	ATCGAACATATCGCGATATGTTCGAT

[a] The top strand is written 5′ → 3′ for the duplexes. Bases enclosed in parentheses indicate that oligonucleotides with a mixture of the indicated bases were synthesized.

Assay of β-galactosidase. Enzyme activity was determined in duplicate or triplicate on at least two different occasions by using logarithmically growing cells permeabilized with sodium dodecyl sulfate and chloroform essentially according to the method described by Miller (15).

RESULTS

Binding of *glp* repressor to consensus and altered operators. In order to determine the nucleotide sequence specificity for the *glp* repressor-operator interaction, a synthetic *glp* consensus operator and various operators with single or symmetrical base substitutions at positions 1 through 9 from the center of operator symmetry were cloned into pGEM3Z. Methods similar to those developed for studying *gal* and *deo* repressor-operator interactions in vivo (9–11) were used to estimate the relative binding affinity of the *glp* repressor for each operator. Strain WO331 [φ(*glpD-lacZ*)*hyb glpR*ⁿ *recA1*] was constructed (Table 1) and employed for characterization of the *glp* repressor-operator interaction. The *glpR*ⁿ allele (noninducible repressor) (6) confers a Lac^- phenotype to this strain because of tight binding of this form of *glp* repressor to the *glpD* operators. β-Galactosidase is produced upon introduction of a multicopy plasmid harboring a *glp* operator because the *glp* repressor is titrated from its binding sites on the chromosome. Thus, the level of β-galactosidase activity obtained from the *glpD-lacZ* fusion (titration level) is proportional to the binding affinity of the repressor for the operator carried by the plasmid. The effects seen are likely due to the influence of the repressor on transcription (and not to posttranscriptional effects), because similar trends were obtained by using the same allele of *glpR* with a transcriptional fusion as the reporter (22). Finally, the levels of β-galactosidase activity obtained in this study are well below the maximum (>5,000 Miller units) produced by this *glpD-lacZ* fusion in a *glpR2* (constitutive) background. Thus, the *glp* repressor is distributed between chromosomal and plasmid operator sites during steady-state growth conditions.

The *glp* repressor bound specifically to the consensus *glp* operator, as indicated by the elevated β-galactosidase activity produced by strain WO331 harboring this operator (120 U) relative to that produced by WO331 (44 U) or by WO331 harboring the vector only (36 U for pGEM3Z [Table 3]). Substitutions at critical positions in the *glp* operator are expected to decrease markedly the binding affinity for the *glp* repressor. Decreased affinity would be reflected by a decreased β-galactosidase activity relative to that found for the strain with the single *glp* consensus operator. Since highly conserved positions of the operator are likely to be critical for binding, *glp*

TABLE 3. Binding of *glp* repressor to consensus and altered operators

Operator name	Sequence[a]	β-Galactosidase sp act[b]
No plasmid		44 ± 3.4
pGEM3Z vector	No operator sequence	36 ± 9.1
Consensus single	TATGTTCGAT ATCGAACATA	120 ± 18
4,4′ A,T (*deo*)	TATGTTAGAT ATCTAACATA	29 ± 4.4
7,7′ A,T	TATATTCGAT ATCGAATATA	17 ± 0.0
7,7′ A,G	TATATTCGAT ATCGAAGATA	20 ± 5.0
7,7′ C,G	TATCTTCGAT ATCGAAGATA	24 ± 6.4
7,7′ C,T	TATCTTCGAT ATCGAATATA	18 ± 1.0
1A	TATGTTCGAA ATCGAACATA	135 ± 6.1
1C	TATGTTCGAC ATCGAACATA	45 ± 5.1
1G	TATGTTCGAG ATCGAACATA	70 ± 5.2
2C	TATGTTCGCT ATCGAACATA	55 ± 2.8
2G	TATGTTCGGT ATCGAACATA	61 ± 5.9
2T	TATGTTCGTT ATCGAACATA	93 ± 1.4
3A	TATGTTCAAT ATCGAACATA	34 ± 4.1
3C	TATGTTCCAT ATCGAACATA	48 ± 2.5
3T	TATGTTCTAT ATCGAACATA	37 ± 2.9
4A	TATGTTAGAT ATCGAACATA	24 ± 1.0
4G	TATGTTGGAT ATCGAACATA	29 ± 1.0
4T	TATGTTTGAT ATCGAACATA	25 ± 1.0
5A	TATGTACGAT ATCGAACATA	23 ± 1.0
5G	TATGTGCGAT ATCGAACATA	23 ± 1.0
5C	TATGTCCGAT ATCGAACATA	30 ± 0.0
6A	TATGATCGAT ATCGAACATA	39 ± 6.5
6G	TATGGTCGAT ATCGAACATA	62 ± 8.3
6C	TATGCTCGAT ATCGAACATA	138 ± 12
7A	TATATTCGAT ATCGAACATA	25 ± 2.2
7C	TATCTTCGAT ATCGAACATA	34 ± 2.2
7T	TATTTTCGAT ATCGAACATA	98 ± 12
8A	TAAGTTCGAT ATCGAACATA	32 ± 1.0
8G	TAGGTTCGAT ATCGAACATA	31 ± 3.0
8C	TACGTTCGAT ATCGAACATA	44 ± 1.0
9G	TGTGTTCGAT ATCGAACATA	81 ± 8.5
9C	TCTGTTCGAT ATCGAACATA	62 ± 7.9
9T	TTTGTTCGAT ATCGAACATA	73 ± 9.2

[a] The mutated bases in the sequence are underlined.
[b] The β-galactosidase specific activities given are the means ± standard deviations derived from at least two independently grown cultures.

operators with alterations at these positions were constructed first. Positions 4 and 7 are the most highly conserved among the native operators (22). When either of these two positions was altered in both half-sites, binding of the repressor was abolished, as indicated by low β-galactosidase activities (<30 U [Table 3]). In order to construct altered operators with less drastic deficiencies in repressor binding, *glp* operators with all possible single substitutions at positions 1 to 9 were constructed. The ability of the variously substituted *glp* operators to titrate the chromosomally encoded *glp* repressor is listed in Table 3. The substitutions at positions 1 to 9 impaired binding of the *glp* repressor to differing degrees, with the exceptions of A at position 1 (1A) and C at position 6. Substitutions at positions 3, 4, 5, and 8 with any other base caused a large decrease in binding affinity, while substitutions at other positions influenced binding of the repressor differentially, depending on the base substituted. Because the β-galactosidase specific activities reflect the binding affinity of the *glp* repressor for the operator carried by the plasmid, the effect of the substitutions on binding affinity was defined as follows: specific activity greater than 110 U, normal; 90 to 110 U, mild; 60 to 89 U, moderate; or less than 60 U, severe effect on binding. By using these criteria, the results in Table 3 can be summarized as shown in Table 4. It is apparent that all substitutions at

TABLE 4. Effect of operator substitution on binding of *glp* repressor[a]

Effect	Substitution at:								
	9A	8T	7G	6T	5T	4C	3G	2A	1T
Normal				C					A
Mild			T					T	
Moderate	G T C			G				G	G
Severe		C A G	C A	A	C G A	G A T	C T A	C	C

[a] The sequence of the half-site *glp* consensus operator is given in the boxhead. The effects of substitutions on the repressor-binding affinity were estimated according to the data given in Table 3 for β-galactosidase specific activity: <60 U, severe; 60 to 89 U, moderate; 90 to 110 U, mild; and >110 U, normal (no effect).

positions 3, 4, 5, and 8 had severe effects on repressor binding, suggesting that these are critical positions for binding of the repressor. Substitutions at position 7 with C or A, at position 6 with A, at position 2 with C, and at position 1 with C also had severe effects on binding. Substitutions at position 9 with any nucleotide, at position 6 with G, at position 2 with G, and at position 1 with G had moderate effects on binding. Operators 7T, 6C, 2T, and 1A yielded high levels of β-galactosidase activity (>90 U), indicating that these base substitutions are tolerable for or have no effect on binding of the repressor. On the basis of the comparison of results with native operators (22) and the results of this study, a modified *glp* operator consensus sequence can be deduced (Fig. 1). The modified half-site consensus (WATKYTCGWW, where W is A or T, K is G or T, and Y is C or T) is derived from both the frequency of occurrence of the bases in the natural operators and the present data showing which substitutions had a mild or no effect on repressor binding.

Binding of *glp* repressor to differently spaced tandem operators. Tandemly repeated operators occur in the native *glpD*, *glpACB*, and *glpFKX* operons (2, 12, 22, 23). Cooperative binding of the *glp* repressor to these operators has been suggested (22). In order to determine if the repressor binds cooperatively to two adjacent operators, a tandem *glp* operator (with two directly repeated 20-bp consensus operators) was constructed. The spacing of the tandem operators was varied between −2 and +6 by using the appropriate insertions for the constructions. A tandem operator with the *deo* consensus sequence (21) was also constructed.

The binding affinity of each operator construct was measured in vivo by using strain WO331 as described above. The results (Table 5) indicate that the repressor binds cooperatively to the tandem *glp* operator, because the β-galactosidase activity was more than 10 times higher in the strain harboring the tandem operators than in the strain with the single consensus operator (1,440 versus 120 Miller units). The insertion of 2 bases between the operators [$(glp)_2$+2] had no effect on cooperativity, as indicated by the high β-galactosidase activity (Table 5). Sequence verification of potential $(glp)_2$+2 clones yielded a derivative with a single base substitution in one of the tandem operators [$(glp)_2$+2(8′G)]. This operator also exhibited full cooperativity for binding of the repressor (Table 5). The deletion of 2 bp or insertion of 4 bp resulted in

Operator	Position	Sequence	% Match with Consensus
O_D1	-10	TATGTTCGAT AACGAACATT	100
O_D2	+11	TATGagCtTT AACGAAAgTg	75
O_A1	-60	AATGTTCaAA ATgacGCATg	75
O_A2	-28	AcTTTcgaAT TAtGAGCgaA	70
O_A3	-8	TATGCgCGAA ATCaAACAaT	85
O_A4	+33	AATGgTaaAA AACGAACtTc	75
O_T	-51	TgTGTgCGgc AAttcACATT	65
O_F1	-89	AtgGCgCGAT AACGctCATT	75
O_F2	-68	TATGacgagg cACacACATT	55
O_F3	-47	TAaGTTCGAT ATttctCgTT	70
O_F4	-27	TtTGCTCGTT AACGAtAAgT	85
O_K1	+993	cgcGgTCGTA ATgGAtCAcg	60
O_K2	+1035	gcaGCgCGAA TTtGAGCAaA	70

Frequency of Occurrence:

$A_{11}A_{15}T_{18}G_{23}T_{11}T_{14}C_{18}G_{15}A_{13}T_{16}$
$T_9\ T_5\ C_4\ T_3\ C_7\ G_{10}A_3\ A_8\ T_{11}A_7$
$C_4\ C_3\ A_3\ \ A_6\ C_2\ T_3\ C_2\ G_2\ G_2$
$G_2\ G_3\ G\ \ G_2\ \ G_2\ T\ \ C$

Operator half-site consensus:

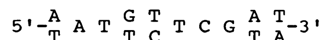
5'-A/T A T G/T T/C T C G A/T T/A-3'

FIG. 1. Comparison of the operators of the *glp* regulon. The operator half-site consensus sequence is based on comparison of native operators and on results of this study. The position number refers to the position of the first base listed relative to the start of transcription. Bases matching the consensus are indicated in uppercase letters. This figure is modified from Fig. 9 in reference 22.

decreased cooperativity, while the insertion of 6 bp eliminated the cooperativity between tandem operators (the ratio of the activities of $(glp)_2$+6 to the single *glp* consensus operator was 2). The *glp* repressor was unable to bind to the $(deo)_2$ operator (β-galactosidase activity was the same as that from WO331 with the vector) although a tandem symmetrical DNA sequence was provided.

DISCUSSION

The *glp* repressor negatively controls the members of the *glp* regulon by binding to the *glp* operator sites. A total of 13 operator sites have been identified by using DNase I footprinting. Sequence comparison revealed a 10-bp operator half-site consensus sequence (Fig. 1) (22). Each operator sequence matches more or less well the consensus sequence. The frequency of occurrence at each operator position is also shown in Fig. 1. Results obtained in the present study showed that the *glp* repressor binds specifically to the consensus operator sequence. Substitutions at positions 1 to 9 resulted in a decreased binding affinity (except for 1A and 6C). The effect was mild, moderate, or severe, depending on the position at which the substitution was introduced and on the specific base used. From the data presented in Table 3, it is apparent that all substitutions at operator position 3, 4, 5, or 8 resulted in almost complete loss of repressor binding, suggesting that these positions are most critical for sequence-specific binding by the repressor. This conclusion is consistent with that predicted from comparison of the native operators (Fig. 1), which clearly shows that positions 3, 4, 5, 7, and 8 are the most highly conserved.

Operator substitutions at positions 7 (A or C), 2 (C), and 1 (C) also had severe effects on repressor binding, while substitutions at positions 1 (G), 2 (T or G), 7 (T) and 9 had mild-to-moderate effects on binding. Position 6 is less well conserved than other positions in the *glp* operator (Fig. 1). The effects of substitutions at this position were quite heterogeneous. The substitution with C had no influence on the binding affinity for the repressor, while 6G or 6A had moderate and severe effects on binding, respectively. All of the results suggest that positions 3, 4, 5, 7, and 8 are most important for sequence-specific binding, while positions 1, 2, 6, and 9 also contribute to the specificity. The magnitude of the effect of each base substitution was consistent with the frequency of occurrence of that base at that position in most cases (Fig. 1).

Substitution of C and G with A and T at positions 4 and 4′, respectively, changes the *glp* consensus operator to the *deo* consensus operator (21). The resulting single *deo* consensus operator or the tandem derivative $[(deoO)_2]$ was not recognized by *glp* repressor. Studies on the sequence specificity of the *deo* operator site by Hammer et al. (10) showed that substitution at 4 and 4′ with C and G (*deo* operator → *glp* operator) also eliminated the specific binding of the *deo* repressor to this modified *deo* operator. Thus, these base differences at positions 4 and 4′ distinguish the *glp* operator from the *deo* operator and allow control of the *glp* and *deo* regulons by their respective repressors.

Even though positions 1 to 9 of the *glp* operator are important for the binding of the repressor, most of the individual native operators deviate significantly from the consensus operator (Fig. 1). Cooperative binding to multiple operator sites clearly provides the basis for repression, which

TABLE 5. Binding of *glp* repressor to tandem operators

Operator name	Sequence	β-Galactosidase sp act[a]
pGEM3Z vector	No operator sequence	36 ± 9.1
Consensus, single	5′-TATGTTCGATATCGAACATA-3′	120 ± 18
deo tandem	5′-TATGTTAGATATCTAACATATATGTTAGATATCTAACATA-3′	36 ± 2.1
glp tandem	5′-TATGTTCGATATCGAACATATATGTTCGATATCGAACATA-3′	1440 ± 80
glp tandem (−2)	5′-TATGTTCGATATCGAACATATGTTCGATATCGAACATA-3′	990 ± 48
glp tandem (+2)	5′-TATGTTCGATATCGAACATATATATGTTCGATATCGAACATA-3′	1500 ± 9.2
glp tandem (+2; 8′G)	5′-TATGTTCGATATCGAACGTATATATGTTCGATATCGAACATA-3′	1530 ± 140
glp tandem (+4)	5′-TATGTTCGATATCGAACATATCGATATGTTCGATATCGAACATA-3′	780 ± 93
glp tandem (+6)	5′-TATGTTCGATATCGAACATATCGCGATATGTTCGATATCGAACATA-3′	220 ± 5.9

[a] The β-galactosidase specific activities given are the means ± standard deviations derived from at least two independently grown cultures.

may greatly increase the responsiveness to control by the repressor. Regulation of transcription of the P_L and P_R promoters of phage lambda is mediated by cooperative binding of the lambda repressor to tandem operators (1, 8). Cooperative binding of the repressors to widely separated operator sites has also been demonstrated in many systems, including the *lac*, *ara*, *gal* and *deo* operons (1, 16). The *glp* repressor apparently utilizes both types of cooperative binding. Repressor-mediated DNA looping has been implicated for control of the divergent *glpTQ-glpACB* operons (12), and the *glpD*, *glpFKX*, and *glpACB* operons each contain tandem operators (12, 22, 23). These latter operons are the most tightly controlled by the *glp* repressor (22). In the present study, direct evidence for cooperative binding of the *glp* repressor to tandem operators was obtained. The ratio of β-galactosidase activity from cells with *glp* tandem $[(glp)_2]$ or *glp* tandem plus 2 bp $[(glp)_2+2]$ operators to the activity from cells with the *glp* single operator was 13, indicative of cooperative binding to the tandem operators. Insertion of 4 bp $[(glp)_2+4]$ or deletion of 2 bp $[(glp)_2-2]$ between the two operators resulted in a decrease in the cooperativity (ratios of β-galactosidase activities in cells with tandem operators to those in cells with a single operator were about 8). The insertion of 6 bp (approximately one-half of a helical turn) between the operators eliminated cooperative binding. In this case, the ratio of β-galactosidase activity (tandem to single) was 2, implying that $(glp)_2+6$ functions like two independent operators.

The $(glp)_2+2$ 8′G operator was obtained by spontaneous mutation in a plasmid carrying the $(glp)_2+2$ operator. Since position 8 is critical for repressor binding, it was anticipated that this operator might exhibit decreased cooperative binding of the repressor. The results indicated that cooperative binding of the repressor was as strong as that of the tandem operator $[(glp)_2]$, suggesting that cooperativity may override absolute sequence specificity for repressor binding. This may explain the tight control of the *glp* operons by the *glp* repressor even when the individual operator sequences do not perfectly match the consensus *glp* operator. This flexibility in operator sequence (Fig. 1) allows overlapping of the operators with the other elements of the *glp* promoters in different ways, which in turn provides another variable parameter facilitating differential control of the *glp* operons.

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