JOURNAL OF BACTERIOLOGY, Jan. 1993, p. 251–258
0021-9193/93/010251-08$02.00/0

Vol. 175, No. 1

Control of Transcription of Gal Repressor and Isorepressor Genes in *Escherichia coli*

MICHAEL J. WEICKERT† AND SANKAR ADHYA*

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Received 6 August 1992/Accepted 30 October 1992

Two regulatory proteins, Gal repressor and isorepressor, control the expression of the *gal* and *mgl* operons in *Escherichia coli*. The transcription start sites for *galR* and *galS*, the genes for the repressor and isorepressor, were determined by primer extension of in vivo transcripts. Study of the promoter-*lacZ* gene fusions introduced into the chromosome indicated that *galS* expression was elevated in cells in which the normal *galS* gene was interrupted, but not in cells in which the *galR* gene was deleted. When both genes were disrupted, *galS* expression was further elevated. Expression from the *galS* promoter was stimulated by the addition of D-fucose, repressed by glucose, and dependent on cyclic AMP receptor protein (CRP). Expression of a similar gene fusion of the *galR* promoter to *lacZ* was unregulated. Both *galR* and *galS* genes contain two potential operator sites (O_E and O_I) and a CRP-binding site. The arrangement of O_E, O_I, and the CRP-binding site in the *galS* gene is analogous to the arrangement in the *gal* and *mgl* promoters, but the arrangement in *galR* is atypical. The increased concentration of the isorepressor when inducer is present may facilitate early shutoff of the isorepressor-regulated genes of the *gal* regulon when inducer (substrate) concentration falls.

In *Escherichia coli*, the genes responsible for D-galactose metabolism (the galactose operon [*galETK*]) and for high-affinity transport of methylgalactosides, glucose, and galactose (the *mglBAC* operon) are controlled by a repressor, GalR (5, 17, 18, 24), and an isorepressor, GalS (9, 32). Both operons are induced by D-galactose or by its nonmetabolizable analog, D-fucose (3, 4, 7, 12, 14, 16, 23, 32). For GalR, inducer binding results in a loss of repressor affinity for DNA (17, 18). The same mechanism is presumed to mediate *mgl* induction, which is under the control of GalS (32).

GalR and GalS have overlapping regulatory specificities, as indicated by ultrainduction of the *gal* operon. In the absence of GalR, the *gal* operon is induced two- to fourfold by the addition of D-galactose or D-fucose (27). Ultrainduction, which occurs at the level of transcription, is mediated by GalS (9, 32), which can be titrated specifically by multiple copies of the same operators to which Gal repressor binds (27).

GalS and GalR belong to the GalR-LacI family of regulators (33). Two of the other members of this family, PurR and CytR, are negatively autoregulated. Negative autoregulation of PurR is mediated by interaction of PurR with two operators downstream of the start of transcription (20, 22). Repression is two- to threefold and requires hypoxanthine or guanine as a corepressor. CytR is also negatively autoregulated by twofold when CytR is supplied on a low-copy-number plasmid to cells containing a fusion of the *cytR* promoter and the *lacZ* gene (8). The *cytR* promoter contains one *cytR* operator upstream of the transcription start site. We investigated whether the Gal repressor and isorepressor are regulated, autoregulated, or cross-regulated. We present evidence that *galS* is (i) negatively autoregulated and (ii) positively regulated by cyclic AMP (cAMP) receptor protein (CRP) and that *galR* is constitutively expressed.

* Corresponding author.
† Present address: Somatogen, Inc., 5797 Central Ave., Boulder, CO 80301.

MATERIALS AND METHODS

Bacterial strains, plasmids, phage, and growth conditions. A list of bacterial strains and plasmids used in this study is presented in Table 1. Bacteriophage P1*vir* was from our laboratory collection, and λNT5 (6) was a gift from N. Trun. The transfer of Δ*galR*::Cm^r, *galS*::Tn*10* (Tc^r), or Δ*crp*::Cm^r to strain MC4100 or its derivatives was by bacteriophage P1-mediated transduction followed by selection for resistance of the transductants to the appropriate antibiotics. Phage λNT5 contains a promoterless, truncated *lacZ* gene and a truncated *bla* gene incapable of conferring resistance to ampicillin (Ap^r). Lysates of λNT5 were prepared with strain TG1 containing either plasmid pPRW201 or pPSW201. Subsequent infection of the MC4100-derived strains containing *galR* and *galS* mutations by these lysates created Ap^r *lac*$^+$ lysogens by homologous recombination between the plasmid and the phage.

The media used for this study were made as described by Miller (21) and by Tokeson et al. (27). Broth cultures were prepared in Luria broth (LB) or minimal medium consisting of M63 supplemented with 0.4% fructose or 0.2% glycerol, 0.1% Casamino Acids, 1 μg of thiamine per ml, and appropriate antibiotics when necessary. Ampicillin was used at a concentration of 50 μg/ml, and tetracycline and chloramphenicol were used at a concentration of 25 μg/ml.

Enzymes and chemicals. Restriction endonucleases, T4 DNA ligase, Moloney murine leukemia virus reverse transcriptase, T4 DNA polymerase, and T4 polynucleotide kinase were purchased from GIBCO BRL (Gaithersburg, Md.), and calf intestinal alkaline phosphatase was obtained from Promega (Madison, Wis.). *O*-Nitrophenyl-β-D-galactoside was obtained from Calbiochem (La Jolla, Calif.); isopropyl-β-D-thiogalactopyranoside was obtained from U.S. Biochemicals, Inc. (Cleveland, Ohio); D-fructose, D-galactose, and D-glucose (dextrose) were obtained from Pfanstiel Laboratories, Inc. (Waukegan, Ill.); and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was obtained from Boehringer Mannheim (Indianapolis, Ind.). Denaturing polyacrylamide gel solutions for sequencing were Gel-Mix

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype	Source or reference
Strains		
MC4100	F⁻ *araD139* Δ(*argF-lac*)U169 *rpsL150 deoC1 relA1 thiA ptsF25 flbB5310*	M. Casadaban
AG701	MC4100 *galE::lacZ galS*::ΔTn*10*/F' *gal*⁺	9
MW130	MC4100 Δ*galR*::Cm^r	This study
MW131	MC4100 *galS*::ΔTn*10* (Tc^r)	This study
MW132	MC4100 Δ*galR*::Cm^r *galS*::ΔTn*10* (Tc^r)	This study
MW134	MC4100 (λNT5 Ap^r *pgalR-lacZ*⁺)	This study
MW136	MC4100 (λNT5 Ap^r *pgalS-lacZ*⁺)	This study
MW138	MW130 (λNT5 Ap^r *pgalR-lacZ*⁺)	This study
MW140	MW130 (λNT5 Ap^r *pgalS-lacZ*⁺)	This study
MW142	MW131 (λNT5 Ap^r *pgalR-lacZ*⁺)	This study
MW144	MW131 (λNT5 Ap^r *pgalS-lacZ*⁺)	This study
MW146	MW132 (λNT5 Ap^r *pgalR-lacZ*⁺)	This study
MW148	MW132 (λNT5 Ap^r *pgalS-lacZ*⁺)	This study
MW157	MW134 Δ*crp*::Cm^r	This study
MW158	MW136 Δ*crp*::Cm^r	This study
SA2600	F⁻ *rpsL his relA*	2
TG1	K-12 Δ(*lac-pro*) *supE thi hsdD5*/F' *traD36 lacI*^{q} *lacZ*Δ*M15*	Amersham Corp.
Plasmids		
pDOG	pBluescript SK+ Δ(*Eco*RI-*Hin*dIII) ::*galS*⁺ Ap^r *lacZ*	32
pGROP	pMT4 Δ(*Eco*RI-*Bam*HI)::[p291Δ (*Eco*RI-*Bam*HI *galO*$_E$⁺*O*$_I$⁺] *galR*⁺ Km^r	32
pMLB1034	Ap^r; promoterless *lacZ*	26
pPRW201	pMLB1034 Ap^r *pgalR-lacZ*⁺	This study
pPSW201	pMLB1034 Ap^r *pgalS-lacZ*⁺	This study

(GIBCO BRL) or Sequagel (National Diagnostic, Manville, N.J.).

Cloning. Basic cloning protocols used were those described by Maniatis et al. (19) and Sambrook et al. (25). Competent cells were prepared and frozen by the method of Hanahan (10).

Polymerase chain reaction (PCR) subcloning of the *galR* and *galS* promoters was performed by using a Gene Machine II (USA/Scientific Plastics, Ocala, Fla.) thermocycler and primers which included unique restriction sites in their sequences. After 24 amplification cycles, the DNA was extracted once with an equal volume of chloroform-isoamyl alcohol (24:1 [vol/vol]) and precipitated with 2 volumes of pure ethanol. After one wash with 70% ethanol, the ends were filled in by using T4 DNA polymerase according to the manufacturer's instructions (GIBCO BRL). After being incubated for 30 min at 37°C, the DNA solution was extracted with an equal volume of chloroform-isoamyl alcohol and the blunt-ended fragments were precipitated by adding 0.1 volume of 7 M sodium acetate and 2 volumes of pure ethanol. The DNA was washed once with 70% ethanol, dried, resuspended in restriction enzyme digestion buffer, and digested with *Eco*RI and *Bam*HI. After digestion, the DNA was loaded into a 3% NuSieve GTG agarose gel (FMC BioProducts, Rockland, Maine). Separation of the promoter fragment from contaminating DNA was accomplished by electrophoresis at 8 V/cm for 45 min. The portion of the gel containing the promoter fragment was removed, and this DNA was ligated to pMLB1034, which had been digested with *Eco*RI and *Bam*HI and then dephosphorylated by using calf intestinal alkaline phosphatase according to the manufacturer's instructions. Ligation was accomplished in the gel in accordance with the manufacturer's instructions. Competent TG1 cells were transformed directly with this ligation mixture, and clones containing PCR promoter fragment inserts grew as blue colonies on LB plates containing 40 μg of X-Gal per ml and 50 μg of ampicillin per ml, while those transformed with vector pMLB1034 alone produced white colonies.

Enzyme assays. Cells were grown and galactokinase assays were performed as described by Adhya and Miller (1). Levels of β-galactosidase were determined from cells grown in M63 minimal medium supplemented with 0.2% glycerol or 0.4% fructose, 0.1% Casamino Acids, and 1 μg of thiamine per ml and permeabilized by the addition of toluene as described by Miller (21).

DNA sequencing. The sequence upstream of the *galR* gene was determined from plasmid pGROP containing a *galR* clone. The sequences of promoter clones were confirmed from plasmid DNA prepared from broth cultures by alkaline lysis followed by passage of the lysate through anion exchange columns by using procedures, solutions, and columns obtained from Qiagen, Inc. (Studio City, Calif.). The DNA was sequenced by using Sequenase version 2.0 (USB, Inc.) reagents and the manufacturer's instructions for double-stranded plasmid sequencing. Sequencing primers (*galR*, 5'-TTAATGACGCGGGAAACGG-3'; *galS*, 5'-AAACCGT TGCCACAGAG-3' and 5'-TGACGCGCTACATCACG-3') were synthesized on an Applied Biosystems 380B DNA synthesizer by using trityl off synthesis, and purified by evaporation with a Savant Speed Vac concentrator, two or three cycles of rehydration in deionized water, and reevaporation.

In vivo transcription. Total *E. coli* RNA was purified from cells growing in LB plus antibiotic, since the promoters examined were present on plasmids. Hot phenol extraction followed by sodium acetate precipitation was performed as described by Lau et al. (15). Purified γ-^{32}P end-labelled sequencing primers and total cell RNA were coprecipitated with ethanol, and primer extension of in vivo transcripts was performed as described previously (32).

RESULTS

Determination of the *galR* and *galS* transcription start sites. We identified the in vivo start sites of transcription for the *galR* and *galS* genes by primer extension with template strand primers from within the coding regions of the genes that were extended through polymerization by Moloney murine leukemia virus reverse transcriptase. mRNA specific for *galR* or *galS* was difficult to detect when the genes were in single copy. To increase the level of *galR* and *galS* specific message, RNA was isolated from strains containing multiple copies of *galR* or *galS* on a plasmid in addition to the normal chromosomal copy. The primer extension products were analyzed by electrophoresis alongside sequencing ladders generated by extension of the same end-labelled primers. Although primer extension can yield bands indicative of RNA processing as well as those indicative transcription start sites, we saw no evidence of message degradation. Figure 1 shows what we believe to be the transcription start sites along with the corresponding DNA sequence from the template strand. Transcription starts at a single position in both promoters, an A (Fig. 2), the complement of T in the template strand sequencing ladder in Fig. 1. In both promoters, transcription begins near the translation initiation codon, 30 bp upstream in *galR* and 42 bp upstream in *galS* (Fig. 2).

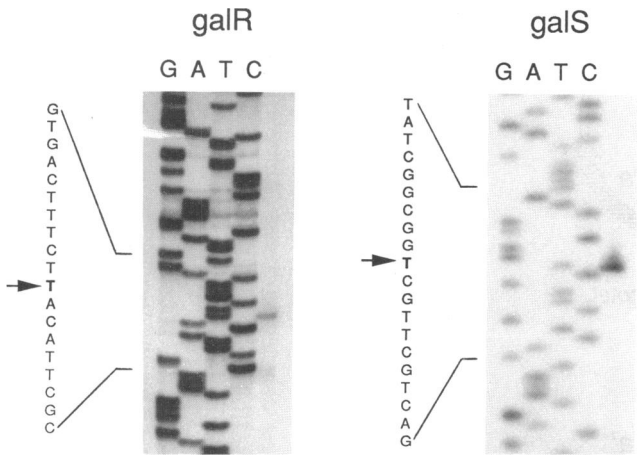

FIG. 1. Transcription from *galR* and *galS* promoters in strains containing multicopy *galS* and *galR* clones. Primer extension of 20 (*galR*) and 50 (*galS*) μg of total RNA per reaction determined the 5′ start sites of transcription. Cells were induced with 4 mM D-fucose for at least 1 h at an optical density at 600 nm of 0.5 to 0.9 before RNA was isolated. The sequencing ladder was generated by using the same end-labelled primer and shows the sequence of the template strand, i.e., the complement of the transcription start site.

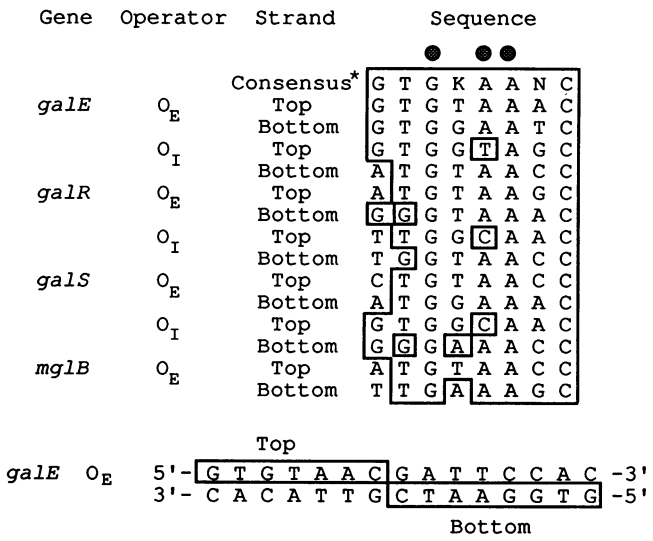

FIG. 3. Comparison of half sites for the symmetric operator sequences and potential operators. The 5′ antisense (left top half) sequence and the 3′ sense (right bottom half) sequence are aligned in a 5′-to-3′ orientation (see $galO_E$ example at bottom). The consensus *gal* operator (18) is at the top. The filled circles above the consensus sequence indicate bases believed to make direct contact with GalR.

Characterization of control elements in *galR* and *galS* promoters. We resequenced the *galR* promoter region and an additional 168 bp 5′ to that previously published (31). Upstream of the transcription start site, the *galR* promoter has a weak σ^{70} promoter. The −10 region contains three of six bases homologous to the TATAAT consensus, separated by 17 bp from the −35 region with four of six bases homologous to the TTGACA consensus (Fig. 2). The *galS* promoter bears little resemblance to the *galR* promoter (Fig. 2). Eight bases 5′ to the transcription start sites is a −10 region with four of six bases homologous to the consensus sequence, but the −35 region most homologous to the consensus sequence (four of six bases) is 21 bp away from −10, a spacing rare in *E. coli* (11).

An examination of the *galR* and *galS* sequences revealed four regions homologous to *gal* and *mgl* operators (two for each) (Fig. 3). In each case, one potential operator is located near the promoter region, called O_E, and a second one called O_I, is located in the structural gene. In addition, potential CRP-binding sites homologous to the consensus sequence (TGTGA-N6-TCACA) were found near each promoter (Fig. 2). In *galR*, the CRP-binding site is centered at +9.5, overlapping the external operator. The CRP-binding site overlaps the −35 region in *galS* (Fig. 2).

Construction of single-copy *galS-lacZ* and *galR-lacZ* gene fusions. We determined the effect of mutations in *galR*, *galS*,

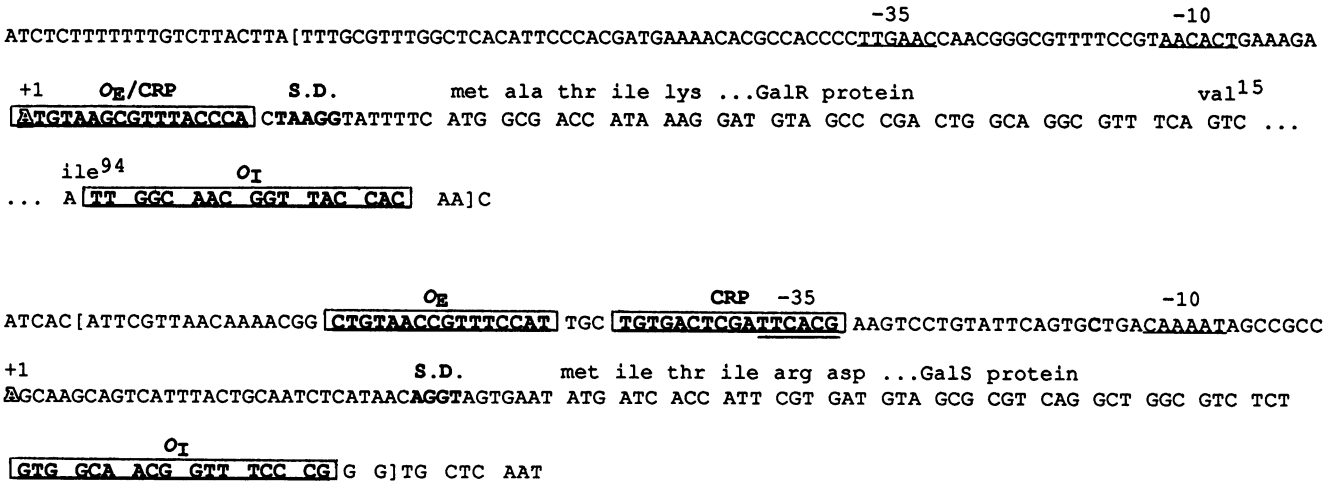

FIG. 2. Sequences of the *galR* and *galS* promoter regions. The sequences of the antisense strand of the *galR* (top) and *galS* (bottom) promoter regions are shown, with important features indicated. The promoter −10 and −35 regions are underlined. The potential Shine-Dalgarno sequence (S.D.) is in boldface type for both *galR* and *galS*. The putative *gal* operators and CRP-binding sites are in boldface type and boxed, and the +1 transcription start sites are indicated by the enlarged letters. The initial codons and translated amino acids of the GalR and GalS proteins are shown. The sequences included in the promoter-*lacZ* gene fusions are enclosed by brackets. Part of the *galR* coding region, from Val-15 to Ile-94, is not shown (· · ·) but is available in reference 31.

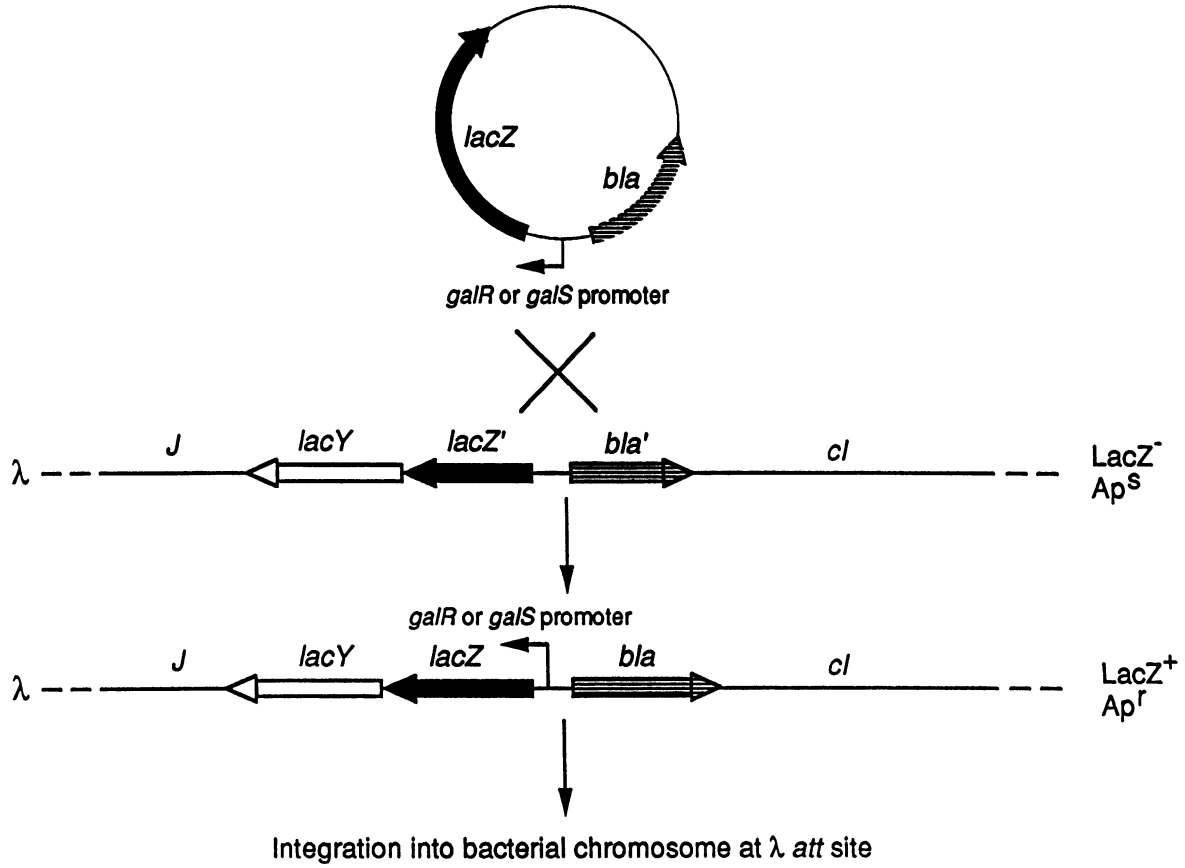

FIG. 4. Strategy for constructing single-copy promoter-*lacZ* fusions. The circular diagram (top) represents plasmids pPRW201 and pPSW201 and contains a fusion of a promoter cloned by PCR (*galR* or *galS* promoter) and the *lacZ* gene. Recombination between the plasmid and homologous sequences on the lambda phage (linear diagrams) is represented by the large ×. A portion of the map of lambda NT5 is shown (middle), with the truncated genes designated *lacZ'* or *bla'*. Lambda NT5 lysogens are LacZ⁻ and ampicillin sensitive (Ap^s). Recombinants (bottom) have intact genes and lysogens and are LacZ⁺ and resistant to ampicillin (Ap^r). Arrowheads indicate the directions of genes; *bla* is the ampicillin resistance determinant.

and *crp* (which encodes CRP) on *galR* and *galS* promoter activity in the presence and absence of inducers (D-galactose and D-fucose). The promoter activities of *galR* and *galS* were determined from single-copy *galS-lacZ* and *galR-lacZ* gene fusions, present in the chromosome as lambda lysogens, by measuring expression of β-galactosidase. The construction of the fusion strains required four steps: creation of Δ*lac* isogenic strains differing only in the *galR*, *galS*, or *crp* gene; making fusions to *lacZ*; construction of an appropriate lambda phage containing the gene fusion; and integration of the phage into the chromosome. The strategy, shown in part in Fig. 4, was carried out as follows.

(i) A series of strains, isogenic except for mutations in the *galR*, *galS*, or *crp* locus, was constructed by bacteriophage P1 transduction into MC4100, a background which is deleted for the chromosomal *lac* operon. Ultrainduction of the *gal* operon was verified by measuring the level of galactokinase, the product of the *galK* gene. In these strains, galactokinase synthesis was derepressed in the strain containing the Δ*galR* mutation but not in the *galS*::Tn*10*-containing strain, as expected (Table 2) (9). Both strains showed ultrainduction in the presence of inducer. The strain carrying both *galR* and *galS* mutations had ultrainduced levels of galactokinase activity with or without the presence of inducer.

(ii) *galR* and *galS* promoter segments (enclosed in brackets in Fig. 2) were amplified by PCR with primers carrying unique restriction sites. After restriction endonuclease digestion, these promoter sequences were ligated to a plasmid containing a promoterless *lacZ* gene, creating translational fusions. Each fusion included the N-terminal portion of the repressor structural gene. This allowed the inclusion of the potential operator sequence found in each of the repressor genes (internal operator [O_I]). The *galR-lacZ* fusion was 94 amino acids longer at the amino terminus than β-galactosidase, while the *galS-lacZ* fusion was 14 amino acids longer. Each clone was confirmed by sequencing.

TABLE 2. Specific activity of galactokinase in isogenic *galS* and/or *galR* mutant strains

Strain	Relevant genotype	Fold induction[a] of galactokinase sp act with:	
		No inducer	D-Fucose
MC4100	Wild type	7.2	74.4
MW130	Δ*galR*	57.8	97.6
MW131	*galS*::ΔTn*10*	14.1	116.0
MW132	Δ*galR galS*::ΔTn*10*	132.1	134.2

[a] Induction was with 4 mM D-fucose for 2 to 3 h.

(iii) Lambda lysates were prepared on strains containing plasmids with *galR*- or *galS-lacZ* promoter fusions. Recombination between the truncated β-lactamase (Ap^r) and β-galactosidase (*lacZ*) genes on the lambda phage and corresponding genes on the plasmids creates a subpopulation of bacteriophage with a complete β-lactamase gene and a β-galactosidase gene transcribed from the cloned promoter (Fig. 4).

(iv) The isogenic strains created in step i above were infected with lysates from step iii. Lambda lysogens resistant to ampicillin and that also produced β-galactosidase, as judged by blue colonies on LB plates containing X-Gal, were selected for further study (Fig. 4).

Effect of *galR* and *galS* mutations on *galS* gene expression. The differential rate of synthesis of β-galactosidase from the *galS-lacZ* fusion was inducible by threefold by D-fucose in a $galR^+$ $galS^+$ or Δ*galR* $galS^+$ background (Fig. 5A and B). In a *galS* mutant, the differential rate of β-galactosidase synthesis was constitutive, equivalent to the fully induced level in the wild type, and was not further inducible by fucose (Fig. 5C). The differential rate of β-galactosidase synthesis was twofold higher still in strains lacking both repressors and was not further inducible (Fig. 5D). This indicates that GalS acts as a repressor of its own synthesis and that GalR represses *galS* in a noninducible manner. The derepression caused by Δ*galR* is epistatic to GalS.

Effect of *galR* and *galS* mutations on *galR* gene expression. Expression of β-galactosidase from the *galR* promoter was not significantly affected by the loss of GalR and/or GalS (Fig. 6), nor was it inducible by D-fucose or D-galactose (data not shown). The *galR* promoter was constitutive under all conditions tested. Although the amount of β-galactosidase produced by the *galR-lacZ* fusion was consistently 5- to 10-fold higher than the amount produced from the *galS-lacZ* fusions, suggesting that the *galR* promoter is stronger than the *galS* promoter, we cannot exclude the possibility that the GalR mRNA or fusion protein is more stable than the corresponding GalS mRNA or fusion protein.

Effect of a *crp* deletion on *galR* and *galS* gene expression. The expression of β-galactosidase from the *galS-lacZ* fusion was reduced by more than 10-fold when strains were grown in minimal medium containing glucose (data not shown), whereas the addition of glucose had only a minimal effect (~25% reduction) on the production of β-galactosidase from *galR-lacZ* fusion strains. Production of β-galactosidase from promoter-*lacZ* fusions in a wild-type strain was compared with that in an isogenic strain into which a deletion of the *crp* gene (with replacement by a Cm^r marker) had been introduced by P1 transduction. Chloramphenicol-resistant *galS-lacZ* transductants were no longer able to synthesize β-galactosidase, as indicated by a lack of colony color on MacConkey lactose or X-Gal plates. When Δ*crp* cells were grown in LB, virtually no β-galactosidase was detected from cells carrying the *galS-lacZ* fusion compared with that detected from the wild type (Fig. 7A). The *crp* deletion had no effect on β-galactosidase production from the *galR-lacZ* fusion (Fig. 7B).

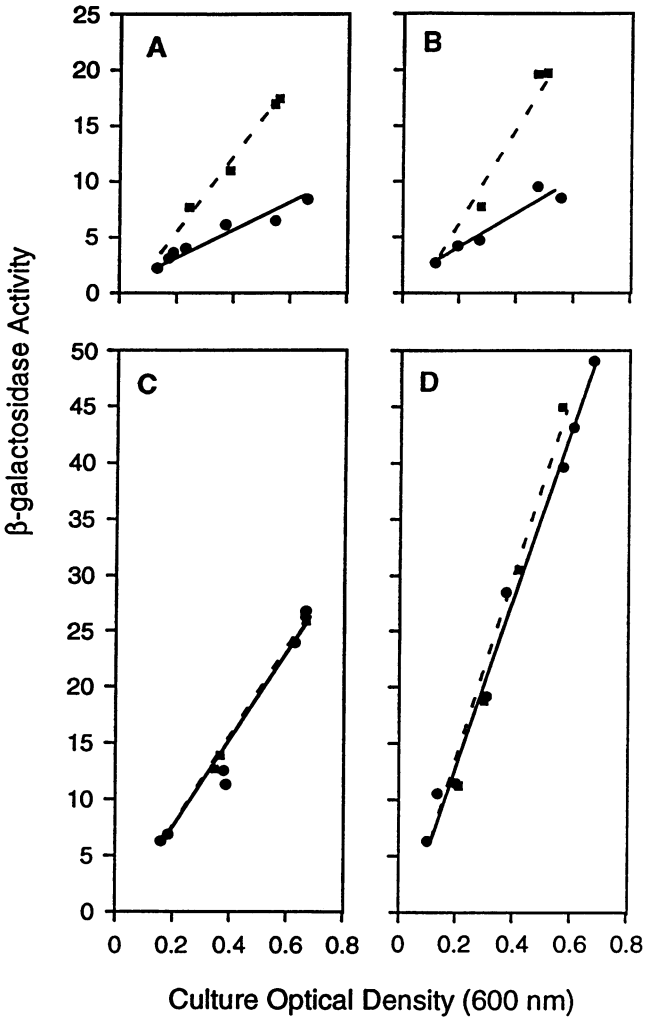

FIG. 5. Differential rate of β-galactosidase synthesis from *galS-lacZ* fusions. Four strains were grown in supplemented minimal medium to an optical density at 600 nm of at least 0.1, at which time the culture was split; half received the inducer D-fucose to a final concentration of 4 mM. Samples were taken throughout exponential growth, and linear regressions of the β-galactosidase activities versus the culture optical density were plotted for each culture. Filled squares and dashed lines indicate cultures supplemented with fucose, and circles and solid lines indicate cultures grown without inducer. (A) Wild type (MW136); (B) Δ*galR* (MW140); (C) *galS* (MW144); (D) Δ*galR galS* (MW148).

DISCUSSION

Features of the *galS* and *galR* promoters. We have determined the in vivo start site of transcription of the *galS* and *galR* genes by primer extension of their mRNAs from strains containing multicopy plasmids with the *galS* or *galR* promoter. The *galR* and *galS* transcripts were virtually undetectable in single copy under the same conditions and RNA preparations used to detect transcripts from the *mgl* and *gal* promoters (32). From the transcription start sites, we have identified sequences likely to be important in the regulation of transcription initiation. The start site of transcription is preceded by a σ^{70}-type promoter in each case. The *galR* promoter and its regulation appear quite simple. The *galR* σ^{70} promoter has a −10 region that is homologous to the TATAAT consensus at only three of six bases, separated by 17 bp from the −35 region homologous to the TTGACA consensus at four of six bases (Fig. 2). The promoter directs *lacZ* expression from our gene fusion in a constitutive manner.

Both promoters contain sequences homologous to *gal* and *mgl* operators (Fig. 3) and CRP-cAMP binding sites. The conservation of the operator sequences suggests a role in the

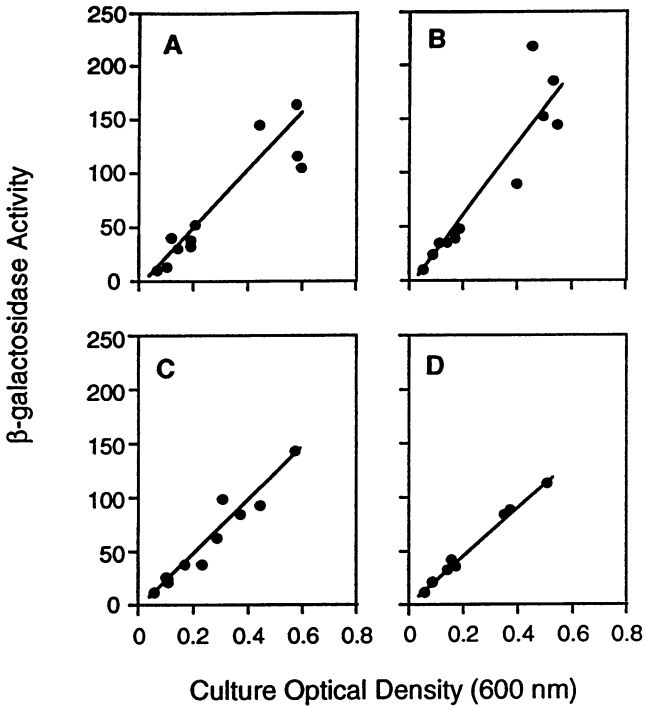

FIG. 6. Differential rates of β-galactosidase synthesis from *galR-lacZ* fusions. Four strains were grown without induction as described for Fig. 5. Datum points are from two independent experiments. (A) Wild type (MW134); (B) Δ*galR* (MW138); (C) *galS* (MW142); (D) Δ*galR galS* (MW146).

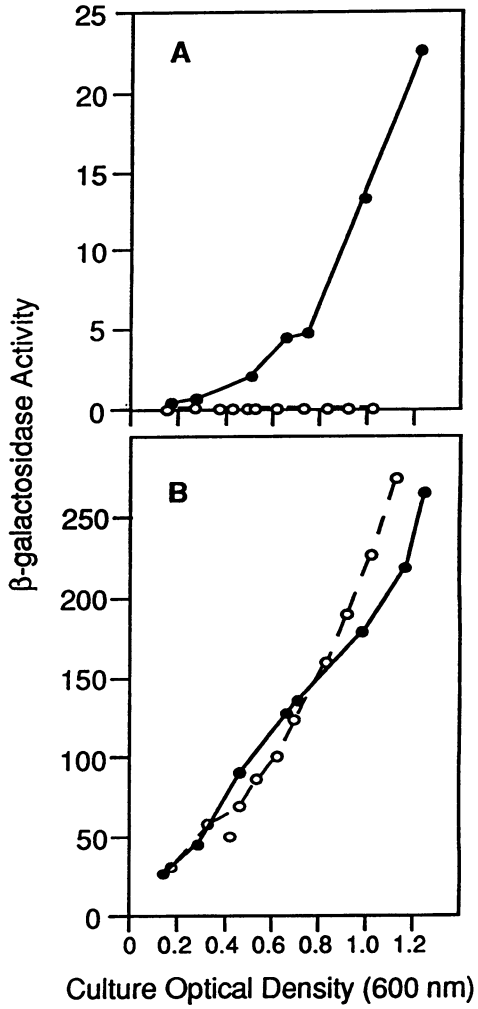

FIG. 7. Differential rates of β-galactosidase synthesis from *galS-lacZ* and *galR-lacZ* fusions in strains containing Δ*crp*. Four strains were grown in LB. Filled circles and solid lines indicate data for *crp*$^+$ strains, and open circles and dashed lines indicate data for Δ*crp* strains. (A) Wild-type *galS-lacZ* (MW136 [filled circles]) and Δ*crp galS-lacZ* (MW158 [open circles]); (B) wild-type *galR-lacZ* (MW134 [filled circles]) and Δ*crp galR-lacZ* (MW157 [open circles]).

negative regulation of these promoters, although this must include a mechanism by which GalR and GalS can discriminate between different operators (32). No function is indicated for the potential operators (O_E and O_I) and CRP-binding sequences in the *galR* promoter region; however, this CRP-cAMP binding site is much less homologous to the CRP-cAMP consensus sequence than are the CRP-cAMP sites in *gal*, *galS*, and *mgl*. In addition, note that the positions of these potential sites with respect to the start site of *galR* transcription (O_E, +8.5; O_I, +318.5; and CRP, +9.5) are different than are for the other *gal* regulon promoters (Fig. 8). We have not distinguished whether the lack of function of these potential regulatory sites is due to the locations or the sequences of the sites. GalR, GalS, and CRP-cAMP apparently do not play any role in *galR* regulation under the conditions we tested.

The *galS* gene also contains a σ^{70}-type promoter, but it is very different from the *galR* promoter. The 7-bp spacer between the −10 region and the transcription start site of *galS* is very G+C rich (Fig. 2). The sequence surrounding the transcription start site is homologous to the discriminator sequence of promoters, which is subject to stringent control (28, 29). The promoters under stringent control are generally expressed at high levels during exponential growth, unlike the *galS* promoter, which is expressed only weakly. The *galS* promoter has unusual spacing between the −10 and −35 regions of the promoter. In *E. coli*, the normal spacing between the −10 and −35 regions is 17 bp (11). The *galS* −10 and −35 regions are 21 bp apart (Fig. 2). The expression of the *galS* promoter is absolutely dependent upon CRP. The scarcity of transcripts as well as the very low expression of β-galactosidase from the *galS-lacZ* fusions, even in the presence of CRP, suggests that it is a poor promoter. However, the −35 sequence of the strong promoter, *galP1*, is separated from the −10 region by 21 bp (13). In addition, both *galS* and *galP1* are CRP-cAMP-dependent promoters whose CRP-binding sites overlap the −35 region. In the *galP1* promoter, this CRP site is centered at −41.5, while for *galS* it is centered at −42.5, but the spacing between the −10 region and the center of the CRP site is 29 bp in each case, a distance optimal for cAMP-CRP-activated promoters in *E. coli* (30). This suggests that the relative weakness of the *galS* promoter, from which no transcripts were detectable compared with the easily detectable transcripts from the *gal* promoter assayed simultaneously, may be due to sequences other than the −10 or −35 sequences (or the spacing between them), such as the G+C-rich discriminatorlike sequence surrounding the transcription start site (Fig. 2).

Negative autoregulation of the *galS* promoter. We have demonstrated that the transcription of the *galS* gene, like the *gal* and *mgl* operons, is inducible by D-fucose and is re-

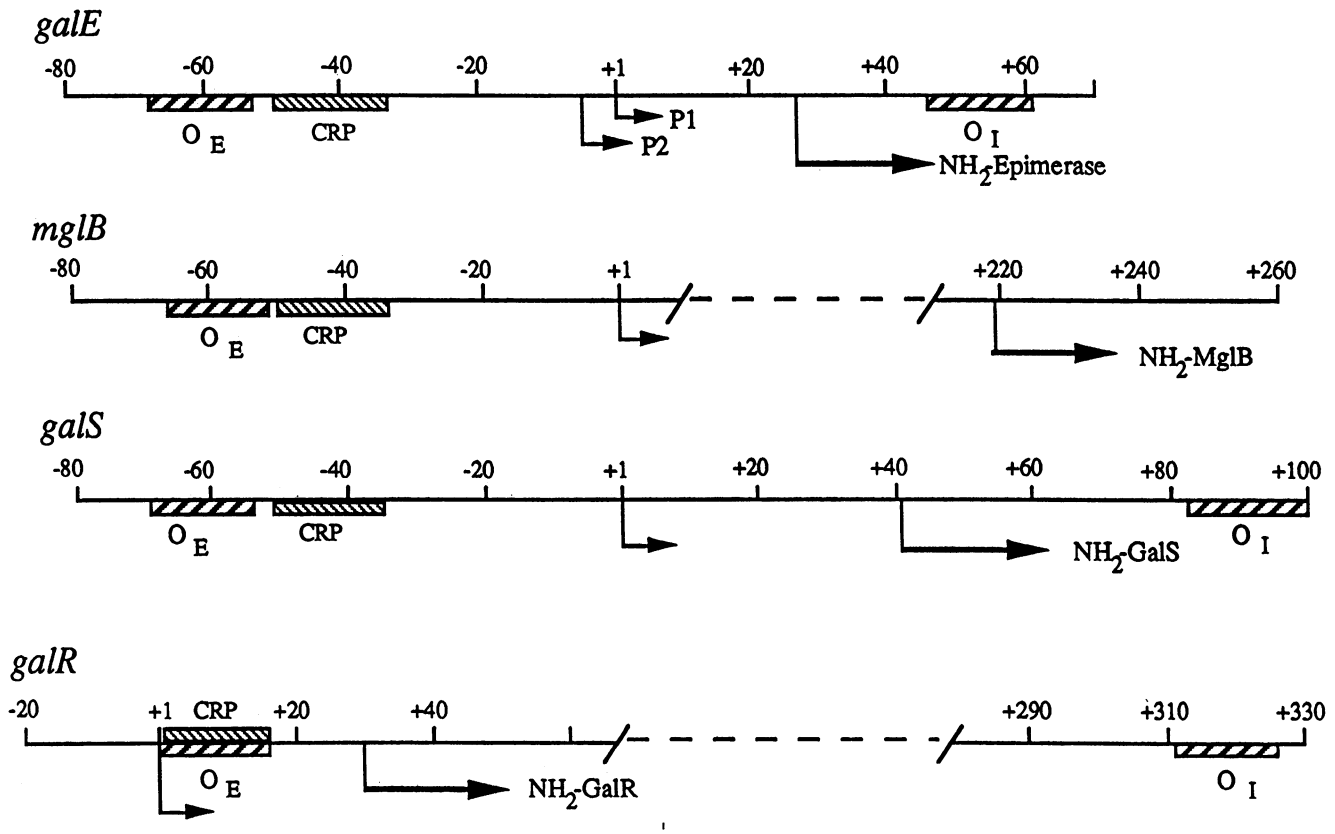

FIG. 8. Comparison of the *galE*, *mgl*, *galS*, and *galR* regulatory sequences. Transcription initiates at +1, indicated by the small arrow, and also initiates at −5 (P2) in *galE*. The beginning of the protein-coding region is indicated by the larger arrow. CRP- and repressor-binding sites (operators) are indicated by hatched boxes. Operator sites are internal (O_I) or external (O_E) to the protein-coding region.

pressed by the product of its own gene. The absence of Gal repressor had no appreciable effect on the differential rate of β-galactosidase synthesis from a *galS-lacZ* fusion unless it was coupled with a *galS* mutation. We have identified a potential external operator, O_E, centered at −61.5 of the *galS* promoter (Fig. 2 and Fig. 8) and an internal one, O_I, centered at +92.5 in the *galS* structural gene (Fig. 8). The arrangement of the two operators is very similar to that of O_E and O_I in the *gal* operon and O_E in the *mgl* operon (Fig. 8). In the *gal* operon, the O_E is centered at −60.5, while for *mgl*, it is centered at −58.5. The *gal* O_I is centered at +53.5, closer to the promoter than it is in *galS* (+92.5). Since GalS apparently binds *gal* or *gal*-like operator sequences (27, 32), we believe that isorepressor (GalS) represses *galS* transcription by binding to O_E or O_I or both.

A model for negative autoregulation. GalS is the third member of the GalR-LacI family of bacterial regulatory proteins for which negative autoregulation has been demonstrated. Negative autoregulation of the expression of PurR, a member of the family, requires two operators, though unlike *galS*, both are located downstream of the start of transcription (20, 22). Negative autoregulation of the expression of CytR, another member of this family, requires only one *cytR* operator located upstream of the transcription start site (8). Like *galS*, repression is weak (two- to threefold) for both *cytR* and *purR*, but PurR requires hypoxanthine or guanine as a corepressor instead of being inducible (22).

Negative autoregulation of these repressors may, in the context of the competing metabolic pathways in a cell, increase a cell's efficiency and flexibility. Small increases in the repressor concentration (two- to threefold) in the presence of inducer do not represent a significant investment, because these regulators are in relatively low abundance. However, the metabolic and transport operons they regulate represent significant commitments of the cell to these pathways. As the substrate (inducer) for these pathways is exhausted, an increased repressor concentration facilitates a quicker shutoff of these operons, avoiding wasteful production of proteins no longer necessary in the cell. This quick adaptation may improve the efficiency and competitiveness of the cell in complex environments.

Implications of *galR* and *galS* regulation for the *gal* regulon. The highly homologous Gal repressor and isorepressor are differentially regulated, suggesting at least some independent functions for each. We have previously demonstrated that the isorepressor is both a second repressor for the *gal* operon and the repressor for the *mgl* operon (32). The evidence presented in this report indicates that the level of *galR* transcription is constitutive, while *galS* transcription is regulated two ways; it has absolute dependence on CRP-cAMP and shows negative autoregulation. We cannot rule out the possibility that *galR* is regulated posttranscriptionally, but since we used a *galR-lacZ* gene fusion, we conclude that GalR is most likely maintained at a constant level in cells. However, maintaining a constant concentration of GalR while carefully regulating the concentration of GalS would be an efficient mechanism of differentially modulating the members of the *gal* regulon to mediate cellular responses to physiological changes.

GalR regulates transcription of the *gal* operon in the

absence of GalS, and GalS regulates transcription of the *mgl* operon (32) and the *galS* gene in the absence of GalR. Ultrainduction of the *gal* operon occurs when either repressor is absent, and the *galS* promoter is sensitive to the loss of both repressors at the same time (Fig. 5), demonstrating that regulation of these promoters includes both repressors. Both GalR and GalS are known to respond to inducers, but the presence of saturating concentrations of inducer does not allow induction of the *gal* operon to the maximal level (27). This may be due to a cellular population of repressor molecules which are multimers composed of a mixture of GalR and GalS subunits. Such heteromers might be resistant to induction and could be considered a third species of *gal* repressor with potentially different characteristics of (i) inducer affinity, (ii) operator affinity, or (iii) ability to undergo the requisite conformational change after inducer binding.

The galactose utilization and transport genes have a system of regulatory control and coordination which includes two repressors as well as the cAMP-CRP complex. We have defined how transcription of the isorepressor gene, *galS*, is autoregulated, while that of *galR* is constitutive. The mechanisms by which they coordinate, with CRP-cAMP, the regulation of the *gal* regulon and discriminate between similar operator sequences could have important implications for the study of global regulatory networks and the regulation of gene expression.

ACKNOWLEDGMENT

M.J.W. is a recipient of an National Institutes of Health Intramural Research Training Award Fellowship.

REFERENCES

1. **Adhya, S., and W. Miller.** 1979. Modulation of the two promoters of the galactose operon of *Escherichia coli*. Nature (London) **279**:492–494.
2. **Adhya, S., and J. A. Shapiro.** 1969. The galactose operon of *E. coli* K-12. I. Structural and pleiotropic mutations of the operon. Genetics **62**:231–247.
3. **Benner-Luger, D., and W. Boos.** 1988. The *mglB* sequence of *Salmonella typhimurium* LT2; promoter analysis by gene fusions and evidence for a divergently oriented gene coding for the *mgl* repressor. Mol. Gen. Genet. **214**:579–587.
4. **Buttin, G.** 1963. Mechanismes regulateurs dan la biosynthese des enzymes du metabolisme du galactose chez *Escherichia coli* K-12. I. La biosynthese induite de la galactokinase e l'induction simultanee de la sequence enzymatique. J. Mol. Biol. **7**:164–182.
5. **Buttin, G.** 1963. Mechanismes regulateurs dan la biosynthese des enzymes du metabolisme du galactose chez *Escherichia coli* K-12. II. Le determinisme genetique de la regulation. J. Mol. Biol. **7**:183–205.
6. **deBoer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1988. Isolation and properties of *minB*, a complex genetic locus involved in correct placement of the division site in *Escherichia coli*. J. Bacteriol. **170**:2106–2112.
7. **Ganesan, A. K., and B. Rotman.** 1965. Transport systems for galactose and galactosides in *Escherichia coli*. I. Genetic determination and regulation of the methyl-galactoside permease. J. Mol. Biol. **16**:42–50.
8. **Gerlach, P., P. Valentin-Hansen, and E. Bremer.** 1990. Transcriptional regulation of the *cytR* repressor gene of *Escherichia coli*: autoregulation and positive control by the cAMP/CAP complex. Mol. Microbiol. **4**:479–488.
9. **Golding, A., M. J. Weickert, J. P. E. Tokeson, S. Garges, and S. Adhya.** 1991. A mutation defining ultrainduction of the *Escherichia coli gal* operon. J. Bacteriol. **173**:6294–6296.
10. **Hanahan, D.** 1985. Techniques for transformation of *E. coli*, p. 109–135. *In* D. M. Glover (ed.), DNA cloning: a practical approach, vol. 1. IRL Press, Oxford.
11. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
12. **Hogg, R. W., C. Voelker, and I. von Carlowitz.** 1991. Nucleotide sequence and analysis of the *mgl* operon of *Escherichia coli*. Mol. Gen. Genet. **229**:453–459.
13. **Irani, M., R. Musso, and S. Adhya.** 1983. Cyclic AMP-dependent switch in initiation of transcription from the two promoters of the *Escherichia coli gal* operon: identification and assay of 5′-triphosphate ends of mRNA by GTP:RNA guanyltransferase. J. Bacteriol. **171**:1623–1630.
14. **Kalckar, H. M., K. Kurahashi, and E. Jordan.** 1959. Hereditary defects in galactose metabolism in *Escherichia coli* mutants. I. Determination of enzyme activities. Proc. Natl. Acad. Sci. USA **45**:1776–1786.
15. **Lau, E., V. Vaughn, and F. Neidhardt.** 1985. Isolation of RNA from *E. coli*. University of Michigan Recombinant DNA Techniques **6**:21.
16. **Lengeler, J., K. O. Hermann, H. J. Unsöld, and W. Boos.** 1971. The regulation of the β-methyl-galactoside transport system and of the galactose binding protein of *Escherichia coli* K12. Eur. J. Biochem. **19**:457–470.
17. **Majumdar, A., and S. Adhya.** 1984. Demonstration of two operator elements in *gal*: *in vitro* repressor binding studies. Proc. Natl. Acad. Sci. USA **81**:6100–6104.
18. **Majumdar, A., and S. Adhya.** 1987. Probing the structure of Gal operator-repressor complexes. J. Biol. Chem. **262**:13258–13262.
19. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Meng, L. M., M. Kilstrup, and P. Nygaard.** 1990. Autoregulation of PurR repressor synthesis and involvement of *purR* in the regulation of *purB*, *purC*, *purL*, *purMN*, and *guaBA* expression in *Escherichia coli*. Eur. J. Biochem. **187**:373–379.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Rolfes, R. J., and H. Zalkin.** 1990. Autoregulation of *Escherichia coli purR* requires two control sites downstream of the promoter. J. Bacteriol. **172**:5758–5766.
23. **Rotman, B., A. K. Ganesan, and R. Guzman.** 1968. Transport systems for galactose and galactosides in *Escherichia coli*. II. Substrate and inducer specificities. J. Mol. Biol. **36**:247–260.
24. **Saedler, H., A. Gullon, L. Fiethen, and P. Starlinger.** 1968. Negative control of the galactose operon in *E. coli*. Mol. Gen. Genet. **102**:79–88.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 250. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Tokeson, J. P. E., S. Garges, and S. Adhya.** 1991. Further inducibility of a constitutive system: ultrainduction of the *gal* operon. J. Bacteriol. **173**:2319–2327.
28. **Travers, A. A.** 1980. Promoter sequence for stringent control of bacterial ribonucleic acid synthesis. J. Bacteriol. **141**:973–976.
29. **Travers, A. A.** 1984. Conserved features of coordinately regulated *E. coli* promoters. Nucleic Acids Res. **12**:2605–2618.
30. **Valentin-Hansen, P., B. Holst, L. Søgaard-Andersen, J. Martinussen, J. Nesvera, and S. R. Douthwaite.** 1991. Design of cAMP-CRP-activated promoters in *Escherichia coli*. Mol. Microbiol. **5**:433–437.
31. **von Wilcken-Bergmann, B., and B. Müller-Hill.** 1982. Sequence of *galR* gene indicates a common evolutionary origin of *lac* and *gal* repressor in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **79**:2427–2431.
32. **Weickert, M., and S. Adhya.** 1992. Isorepressor of the *gal* regulon in *Escherichia coli*. J. Mol. Biol. **226**:69–83.
33. **Weickert, M., and S. Adhya.** 1992. A family of bacterial regulators homologous to Gal and Lac repressors. J. Biol. Chem. **267**:15869–15874.