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Positive and Negative Regulation of the *bgl* Operon in *Escherichia coli*

SUBRAMONY MAHADEVAN, ANN E. REYNOLDS,† AND ANDREW WRIGHT*

Department of Molecular Biology and Microbiology, Tufts University Health Sciences Campus, Boston, Massachusetts 02111

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We have analyzed the functions encoded by the *bgl* operon in *Escherichia coli* K-12. Based on the ability of cloned regions of the operon to complement a series of Bgl⁻ point mutations, we show that the three *bgl* structural genes, *bglC*, *bglS*, and *bglB*, are located downstream of the regulatory locus *bglR* in the order indicated. Using a *bgl-lacZ* transcriptional fusion, we show that *bglC* and *bglS* are involved in regulating operon expression. The presence of the *bglC* gene in *trans* is absolutely required for the expression of the fusion, which is constitutive when only the *bglC* gene is present. When the *bglC* and the *bglS* genes are both present in the cell, expression of the fusion requires a β-glucoside inducer. From these observations, we conclude that (i) the *bglC* gene encodes a positive regulatory of *bgl* operon expression and (ii) the *bglS* gene encodes a negative regulator of operon expression, causing the requirement for a β-glucoside inducer. These conclusions are supported by our observations that (i) a majority of *bglC* mutants exhibits a Bgl⁻ phenotype, whereas rare *trans*-dominant mutations in *bglC* result in constitutive expression of the *bgl* operon and the fusion, and (ii) mutations in the *bglS* gene lead to constitutive expression of the fusion. Based on several lines of evidence presented, we propose that the *bglS* gene product has an additional role as a component of the β-glucoside transport system.

The *bgl* operon in *Escherichia coli* K-12 specifies the enzymes involved in the catabolism of aromatic β-glucosides such as arbutin and salicin. The operon is cryptic and uninducible in wild-type strains, and therefore wild-type cells are unable to utilize salicin or arbutin as a carbon source. Several classes of mutations have been shown to activate the cryptic operon. The major class of activating mutations is effective in *cis* and has been characterized as insertions of IS*1* or IS*5* or point mutations that map within the regulatory locus *bglR* (9). The insertion sequences and point mutations have been shown to enhance transcription from the unique *bgl* promoter, present in the *bglR* region, which is active at a low level in wild-type cells (10). Transcriptional activation, however, does not result in constitutive expression of the operon. After activation, induction of the operon requires the presence of a β-glucoside sugar. Expression of the operon is also subject to catabolite repression.

In addition to the *bglR* site, three structural genes of the *bgl* operon were reported earlier (7). The *bglB* gene was shown to encode the enzyme phospho-β-glucosidase B, which hydrolyzes phosphosalicin. The structural gene encoding the β-glucoside transport system I, the *bgl*-specific component of the phosphoenol pyruvate-dependent phosphotransferase system (2), was identified by measuring phospho-β-glucosidase B activity in cell extracts from Bgl⁻ mutants. A *trans*-dominant mutation, which led to constitutive *bgl* expression, suggested the presence of a third *bgl* gene. Based on the unique phenotype of the constitutive mutation, it was postulated that expression of phospho-β-glucosidase B and the β-glucoside transport system is under positive control. Initial mapping of the *bgl* genes suggested that the genes encoding the hydrolytic and transport functions are located on either side of the *bglR* site, forming divergent transcriptional units.

An unlinked locus termed *bglA*, which specifies a second phospho-β-glucosidase, has been identified (8). This enzyme, termed phospho-β-glucosidase A, preferentially cleaves phosphoarbutin and is produced constitutively by wild-type cells. However, wild-type cells are unable to utilize arbutin due to lack of expression of the β-glucoside transport system I of the *bgl* operon. Therefore, arbutin utilization also requires an activated *bgl* operon for uptake and phosphorylation of the substrate.

The results presented in this paper confirm the presence of three structural genes in the *bgl* operon. We show that the three genes *bglC*, *bglS*, and *bglB* are contained within a 5.7-kilobase (kb) region downstream from the transcription start site in *bglR* characterized previously (10). This operon structure, indicated by our observations, differs from the structure proposed earlier (7). Based on studies with a *bgl-lacZ* transcriptional fusion, we show that the first gene in the *bgl* operon, *bglC*, encodes a positive regulator of *bgl* operon expression. We also show that the second gene, *bglS*, encodes a negative regulator of the *bgl* operon. However, loss of *bglS* gene function simultaneously results in a Bgl⁻ phenotype, suggesting that the *bglS* gene product also has a direct role in β-glucoside utilization. We propose that the *bglS* gene product, in addition to being a negative regulatory of *bgl* operon expression, is the *bgl*-specific component of the phosphoenolpyruvate-dependent phosphotransferase system. Specific roles of the *bglC* and *bglS* genes in the regulation of the operon are considered.

MATERIALS AND METHODS

Strains. The *E. coli* K-12 strains used in this study are listed in Table 1. AE10 is a spontaneous Bgl⁺ derivative of

* Corresponding author.
† Present address: Department of Genetics, University of Washington, Seattle, WA 98105.

TABLE 1. *E. coli* strains, bacteriophages, and plasmids[a]

Strain, phage, or plasmid	Genotype	Source or reference
E. coli		
AE10	F⁻ Δ*lacX74 thi bglR11* (*bglR*::IS*1*) (Bgl⁺)	
AE304	As AE10 and *tsx* (T_6)ʳ (Bgl⁺)	
AE304-1	As AE304 and *tna*::Tn*10 bglR bglS1*	
AE304-2	As AE304 and *tna*::Tn*10 bglR bglS2*	
AE304-3	As AE304 and *tna*::Tn*10 bglR bglB3*(Am)	
AE304-4	As AE304 and *tna*::Tn*10 bglR bglS3*	
AE304-6	As AE304 and *tna*::Tn*10 bglR bglB6*	
AE304-7	As AE304 and *tna*::Tn*10 bglR bglC7*(Am)	
AE304-9	As AE304 and *tna*::Tn*10 bglR bglC8*	
AE304-10	As AE304 and *tna*::Tn*10 bglR bglC10*	
AE325	F⁻ *bglR trpB proC*::Tn*5 ilvO tna-5* (Bgl⁺)	
JF50	F⁻ Δ*lac mel gyrA supF*	J. Felton
JF201	F⁻ Δ*lacX74* Δ(*bgl-pho*)*201 ara thi gyrA*	10
MA10	As AE10 and *srl*::Tn*10 recA56* (Bgl⁺)	
MA46	As MA10 and *bglR*::IS*1 bglB*::Tn*5 bglC4*	
MA46-200	As MA46 and λ *bglR7 bglC' lacZ*⁺ *lacY*⁺ Φ(*bgl-lac*)	
MA110	As AE10 *tna* and Δ(*bgl-pho*) *srl*::Tn*10 recA56*	
MA152	As MA110 and λ *bglR7 bglC' lacZ*⁺ *lacY*⁺ Φ(*bgl-lac*)	
MA200	As MA10 and λ *bglR7 bglC' lacZ*⁺ *lacY*⁺ Φ(*bgl-lac*) (*bgl*⁺)	
MA200-1	As MA200 and *bglS201*	
MA200-2	As MA200 and *bglS202*	
MA200-3	As MA200 and *bglS203*	
MA200-4	As MA200 and *bglS204*	
MA200-33	As MA200 and *bglC33* (Bglᶜ)	
MA221	As AE325 and *tna*⁺ *recA56 lac bglC21*	
MA222	As AE325 and *tna*⁺ *recA56 lac bglC22*	
MA223	As AE325 and *tna*⁺ *recA56 lac bglB23*(Ts)	
MA225	As AE325 and *tna*⁺ *recA56 lac bglB25*	
MA226	As AE325 and *tna*⁺ *recA56 lac bglB26*(Ts)	
MA227	As AE325 and *tna*⁺ *recA56 lac bglB27*	
MA229	As AE325 and *tna*⁺ *recA56 lac bglB29*	
MA231	As AE325 and *tna*⁺ *recA56 lac bglS31*	
MA233	As AE325 and *tna*⁺ *recA56 lac bglC23*	
MA234	As AE325 and *tna recA56 lac bglB34*	
SP3	F⁺ *bglR1* (*bglR*::IS*1*) *bglC4 lamB* (Bglᶜ)	7
WP72	F⁻ *galE arg pro thi rpsL tna*::Tn*10 bglR* (Bgl⁺)	W. Peters

Continued

TABLE 1—*Continued*

Strain, phage, or plasmid	Genotype	Source or reference
Bacteriophages		
λNF1955	λ *cI857* λ *Sam100 lacZ' lacY*⁺	14
λMN200	λ c⁺ *S*⁺ *bglR7 bglC' lacZ*⁺ *lacY*⁺ Φ(*bgl-lac*)	
Plasmids		
pAR6	*bglR*⁰ *bglC*⁺ *bglS*⁺ *bglB*⁺ (Bgl⁻) *glmS*⁺ *phoS*⁺ *phoT*⁺ *phoU*⁺ Apʳ Tcʳ	10
pAR7	As pAR6 and *bglR7* (*bglR*::IS*5*) (Bgl⁺)	
pAR8	As pAR7 Δ(*glm-pho*-IS*5'*) (Bgl⁺)	
pAR10	*bglR7 bglC'* Apʳ	
pAR16	*bglR1 bglC4 bglS*⁺ *bglB*⁺ Apʳ Tcʳ (Bglᶜ)	
pAR18	*bglR7 bglC*⁺ *bglS*⁺ *bglB'*Apʳ	
pMBO41	*trpA'-lac'Z lacY*⁺ *lacA'* Apʳ	3
pMN5	*bglC' bglS*⁺ *bglB'* Apʳ	
pMN25	*bglR25 bglC*⁺ *bglS'* Apʳ	
pSAL6	*bglR3* (*bglR*::IS*1*) *bglC*⁺ *bglS*⁺ *bglB*⁺ Apʳ Tcʳ (Bgl⁺)	
p1H	*bglC' bglS*⁺ *bglB*⁺	
p6J	*bglS' bglB*⁺	

[a] Strains, bacteriophages, and plasmids for which no reference has been cited were constructed as part of this work. Phenotypes of Bgl⁺ strains are indicated in parentheses.

the laboratory strain RV. The activating mutation present in AE10 was characterized as an IS*1* insertion in *bglR*. AE304, a derivative of strain AE10, was used as the parent strain for transducing one class of *bgl* mutations from WP72 after localized mutagenesis (described below). The mutations were transduced by using a Tn*10* insertion in the *tnaA* gene. The *bgl* operon can be cotransduced with *tna* at about 80% efficiency. Strains MA221 through 234 were derivatives of strain AE325 carrying a second set of *bgl* mutations transduced from WP72 after mutagenesis as in the previous case. The *recA56* allele was introduced by using a Tn*10* insertion in *srl* after transducing the *tna*::Tn*10* mutation to *tna*⁺.

The strain JF201, which carries a deletion of the chromosomal *bgl* operon and the adjacent *phoUT* genes, was described previously (10). The *phoUT* deletion results in the constitutive synthesis of alkaline phosphatase encoded by the *phoA* gene. The Phoᶜ phenotype can be suppressed by plasmids carrying the *phoUT* genes. Strain MA110 was derived by transducing the Δ(*bgl-pho*)*201* mutation from JF201 into AE10 with the *tna*::Tn*10* marker. Tetʳ transductants were screened for a Bgl⁻ Phoᶜ phenotype. The *recA56* allele was introduced after selecting for Tetˢ clones (1). Strain MA46 was constructed by transducing the *bglC4* allele from strain SP3 with a Tn*5* insertion in *bglB*. The presence of the *bglC4* allele was confirmed by screening Kanʳ transductants for an Arbᶜ phenotype as described below.

Plasmids. Construction of the plasmids pAR6 and pSAL6 has been described previously (10). Plasmid pAR7, a spontaneous Bgl⁺ derivative of pAR6, contains an IS*5* insertion in *bglR*. Plasmids pAR10 and pAR18 were generated by deleting sequences from pAR7 with *Hin*dIII restriction sites. The *bglC*⁺ plasmid pMN25 was constructed by subcloning the 0.5-kb *Hin*dIII-*Hpa*I restriction fragment from pSAL6

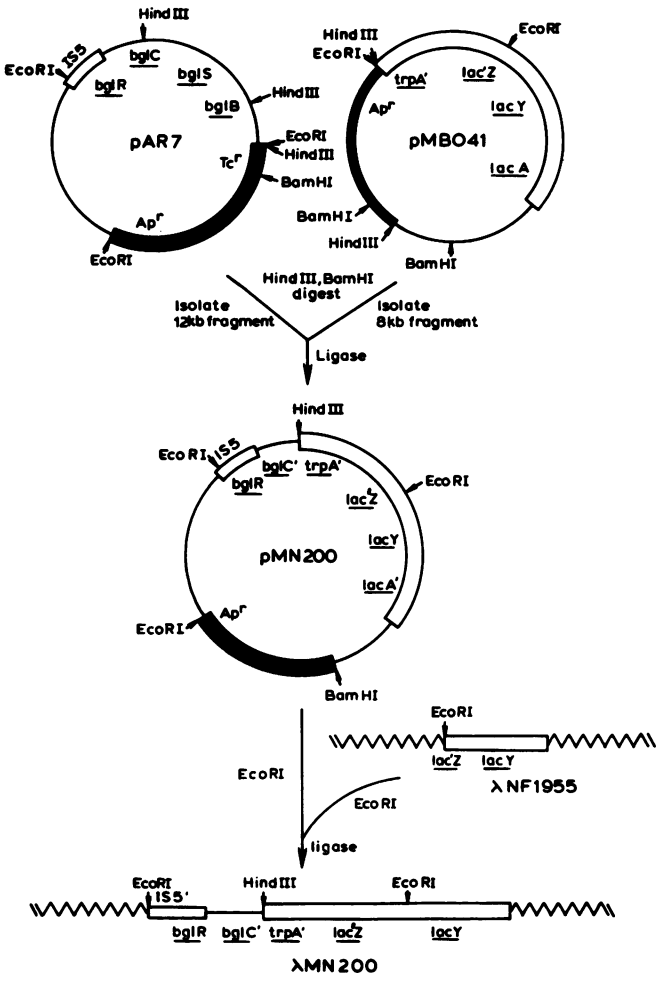

FIG. 1. Construction of a *bgl-lacZ* transcriptional fusion. Symbols: □, activating IS*5* insertion in *bglR*; ■, pBR322 sequences; ∧∧ λ sequences. Plasmid pMBO41 is a derivative of pMBO40 (3) in which a 205-base-pair *Eco*RI restriction fragment carrying the *lacUV5* promoter has been deleted.

downstream of the unique *Hin*dIII site in plasmid pAW25 (10). This construction reconstitutes the *bgl* sequences from the *bglR* site to the second *Hpa*I site in the operon (see Fig. 3).

The plasmid p1H was constructed by partial digestion of pSAL6 DNA with *Hin*dIII and subsequent ligation. The *bglS*$^+$ plasmid pMN5 was derived by subcloning a 2.3-kb *Hin*dIII-*Cla*I restriction fragment from pSAL6 into the *Hin*dIII-*Cla*I site of pBR322. *Hpa*I restriction sites in pSAL6 were used to delete *bgl* sequences to generate the *bglB*$^+$ plasmid p6J. In these three constructions, the *bgl* sequences are located downstream of a promoter in the *tet* gene of pBR322 (15).

The plasmids pAR16 and pMN33, carrying the *bglC4* and *bglC33* alleles, respectively, were derived by transferring the *bglC* mutations from the chromosomes of the parent strains to a *bgl* plasmid as described earlier (10). Hybrid plasmids containing wild-type and mutant sequences, used in the mapping of the two *bglC* alleles, were constructed as shown in Fig. 4.

Media. Minimal and enriched media for routine use were prepared from standard recipies (6). Media used for growing strains carrying pBR322-derived plasmids contained 100 μg of ampicillin per ml. MacConkey arbutin and salicin media were prepared as described previously (11).

Localized mutagenesis of the *bgl* operon. The Bgl$^-$ mutants employed in the genetic analysis of the *bgl* operon were generated by localized mutagenesis of the *bgl* operon region. The Bgl$^+$ parent strain WP72, which carries a Tn*10* transposon in the *tnaA* gene (80% cotransducible with *bgl*), was mutagenized with nitrosoguanidine (6). Bacteriophage P1 was grown on the mutagenized strain, and the resulting lysate was used to transform a Bgl$^+$ strain to tetracycline resistance. Tet^r transductants were screened for the simultaneous acquisition of a *bgl* mutation. In this way we were able to enrich for mutations in the *bgl* operon. Eighteen Bgl$^-$ mutants isolated from 664 Tet^r transductants were used for further analysis. Mutations in the *bglB* gene were identified by their characteristic Arb$^+$ Sal$^-$ phenotype. Temperature-sensitive and amber mutations were recognized by the suppression of the Bgl$^-$ phenotype under permissive conditions.

Molecular cloning. All manipulations with recombinant DNA were carried out by standard procedures (5). Restriction enzymes and other enzymes used in recombinant DNA experiments were purchased commercially and were used according to the specifications of the manufacturers.

Construction of a *bgl-lacZ* transcriptional fusion. The *bgl-lacZ* transcriptional fusion used in these studies was constructed in vitro by fusing sequences containing an IS*5*-activated *bgl* promoter and a portion of the *bglC* gene to *lacZ*. This construction strategy is outlined in Fig. 1. A 12-kb *Hin*dIII-*Bam*HI fragment containing the *bgl* sequences and the pBR322 vector, derived from plasmid pAR7, was isolated and ligated with an 8-kb *Hin*dIII-*Bam*HI fragment containing the *lacZYA* genes derived from the plasmid pMBO41 (3). The ligated DNA was used to transform the Bgl$^+$ strain MA10. Amp^r transformants were screened on minimal succinate medium containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), the chromophoric substrate for β-galactosidase, and 10 mM β-methyl glucoside as inducer. The presence of the correct fusion was verified by restriction analysis of plasmid DNA isolated from transformants that gave rise to blue colonies. The *bgl-lacZ* fusion was transferred from the plasmid pMN200 to the λ vector NF1955 (11) by ligating *Eco*RI digests of the plasmid and λ NF1955 DNA. The ligated DNA was packaged in vitro with λ packaging extracts (11). Phages carrying the *bgl-lacZ* fusion gave pale blue plaques on X-gal plates with a lawn of an Su$^+$ host deleted for the chromosomal *lac* operon. The λ cI857 and the λ Sam100 mutations, present in the original isolates, were replaced with the respective wild-type alleles by crossing the phage with wild-type λ phage. The recombinant phages were recognized by their ability to form turbid blue plaques on X-gal plates at 37°C with an Su$^-$ host.

Measurements of β-galactosidase activity. Assays for β-galactosidase activity were carried out as described previously (6). Cells were grown in minimal medium containing 0.4% succinate as the carbon source. Average values of units of activity were computed based on at least four independent measurements in each case.

Screening for constitutive expression of the *bgl* operon. Constitutive expression of the *bgl* operon was detected by using *para*-nitrophenyl-β-glucoside as a substrate (7). Strains to be tested were grown on minimal succinate plates at 37°C for 24 h. One drop of a 4×10^{-2} M solution of *p*-nitrophenyl-β-glucoside was placed on the patches of cells at room temperature. Strains that expressed the *bgl* operon constitutively developed a bright yellow color in 60 s due to

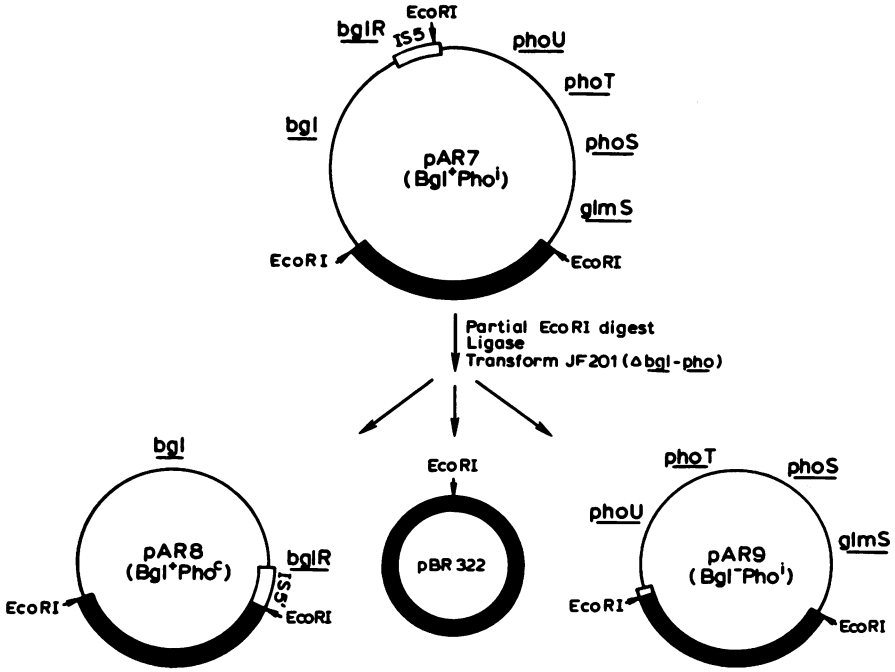

FIG. 2. Mapping of the *bglR* site relative to the structural genes of the *bgl* operon. Symbols: □, activating IS*5* insertion present in pAR7; ■, pBR322 sequences. Phenotypes of the resulting plasmids were tested in strain JF201 (*Δbgl-pho-201*).

the cleavage of the phosphorylated substrate, which liberated the chromophore *p*-nitrophenol.

RESULTS

Location of the *bgl* genes relative to the *bglR* site. The *bglR* region contains the transcription start site and the target sites for insertion sequences and point mutations that activate the *bgl* operon (10). Earlier genetic analysis with three-factor cotransductional crosses had suggested that the *bglR* site is located between two *bgl* structural genes (7). Based on this result, it was proposed that the *bgl* structural genes are transcribed bidirectionally from *bglR*.

We determined the location of the *bgl* structural genes with respect to the *bglR* site by subcloning DNA located on either side of the *bglR* site. *Eco*RI restriction fragments from the Bgl$^+$ plasmid pAR7, which contains an *Eco*RI site in the activating IS*5* element in *bglR*, were subcloned in pBR322 and analyzed for *bgl* function (Fig. 2). One of the resulting plasmids, pAR8, which contained 1.1 kb of the activating IS*5* insertion and 5.7 kb of DNA downstream of the *bgl* promoter in *bglR* (10), conferred a Sal$^+$ Arb$^+$ phenotype on strains deleted for the chromosomal *bgl* operon. Expression of the *bgl* operon in pAR8 was inducible by β-glucosides and required cyclic AMP binding protein and cyclic AMP. The 6.9-kb *Eco*RI restriction fragment conferred an identical phenotype when present in single copy in the chromosome as part of a λ prophage. These results indicate that the *bgl* structural genes are contained within the 5.7-kb region downstream of the *bglR* site.

Mapping of *bgl* mutations. To analyze the functions encoded by the *bgl* operon, we isolated a series of Bgl$^-$ mutants by using localized mutagenesis as described in Materials and Methods. The locations of 18 *bgl* mutations were determined by complementation analysis with a series of plasmids containing different portions of the *bgl* operon (Fig. 3). Plasmids pAR10, pAR18, and pMN25 were derived from Bgl$^+$ plasmids and contain an activated *bgl* promoter. Plasmids p1H, pMN5, and p6J are deleted for the *bglR* site but still express the cloned *bgl* genes, presumably from a promoter within the *tet* gene of the pBR322 vector (15). Transformants of the various Bgl$^-$ mutants carrying these deletion plasmids were screened on MacConkey salicin plates for complementation. Positive complementation was indicated by the formation of bright red colonies. The results of these studies (Table 2) indicate that the *bgl* mutations fall into three groups based on complementation by specific *bgl* plasmids. The properties of each group of Bgl$^-$ mutants are described below.

The *bgl* mutations in strains AE304-7, -9, and -10 and MA221, 222, and 233 were complemented strongly by plasmid pMN25 (complementation of AE304-10 and MA222 was weaker compared with the others). Plasmid pAR10, which carries DNA up to the first *Hin*dIII site in the operon, gave only partial complementation of these mutants as indicated by the formation of pale pink colonies on MacConkey salicin plates. These results indicate that a gene, which we have designated *bglC*, extends beyond the first *Hin*dIII site but does not extend beyond the second *Hpa*I site downstream of the *bgl* promoter (Fig. 3). The partial complementation observed with pAR10 is likely to be due to a low level of activity of the truncated *bglC* gene product, when expressed from a multicopy plasmid.

The *bgl* mutations in strains AE304-1, -2, and -4 and MA231 were complemented strongly by plasmid p1H, but not by p6J. Therefore, these mutations define a gene, which we designate *bglS*, that starts within the region downstream of the first *Hin*dIII site and upstream of the second *Hpa*I site.

A third set of *bgl* mutations, present in strains AE304-3 and -6 and MA223, 225, 226, 227, 229, and 234, were complemented by both p1H and p6J but not by pAR18 or pMN25. These strains are characterized by an Arb$^+$ Sal$^-$ phenotype. Hence, these mutations define the *bglB* gene, the

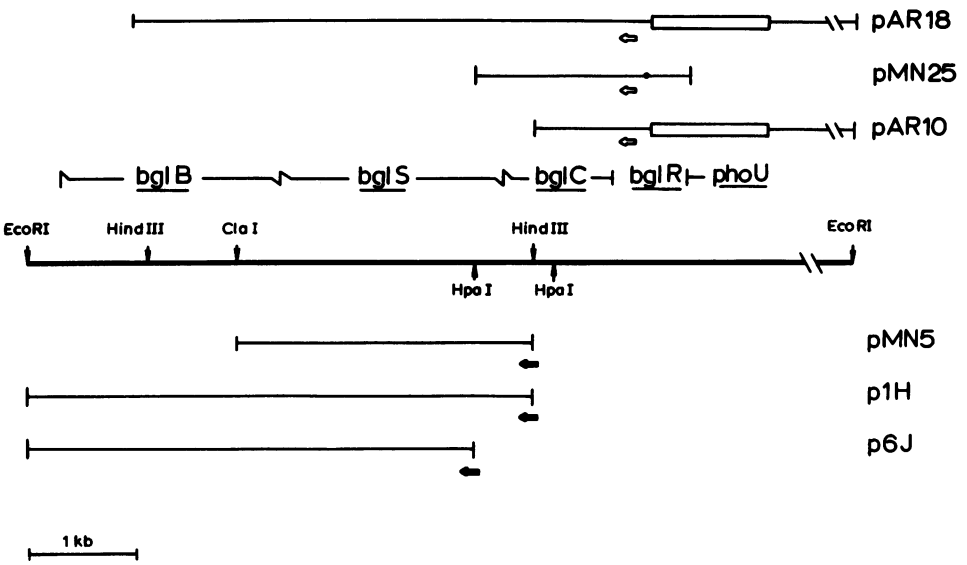

FIG. 3. Structural organization of the *bgl* operon. Chromosomal DNA is represented as a solid line in the center. Plasmids used in the complementation analysis of the Bgl$^-$ mutants are indicated above and below the chromosomal DNA. For simplicity, vector sequences have been omitted. Symbols: □, activating IS*5* insertion in *bglR*; ●, point mutation in *bglR*; ◊, transcription from the *bgl* promoter; ◆, transcription from the pBR322 vector.

structural gene encoding the enzyme phospho-β-glucosidase B, which preferentially cleaves phosphosalicin (7). Strains with *bglB* mutations can utilize arbutin, by using the unlinked locus *bglA* (8), and the β-glucoside transport system I, specified by the *bgl* operon (7). Plasmid pAR18, which carries *bgl* DNA up to the second *Hin*dIII site in the operon (Fig. 3), confers an Arb$^+$ Sal$^-$ phenotype to cells deleted for the chromosomal *bgl* operon, indicating that this *Hin*dIII site lies within the *bglB* gene.

Plasmid pMN5, which carries DNA from the first *Hin*dIII site to the *Cla*I site (Fig. 3), complemented the *bglS* mutations described above, resulting in an Arb$^+$ phenotype. However, these transformants had a Sal$^-$ phenotype, suggesting that the *bglS* mutations tested were polar on the *bglB* gene. The same results were obtained when these *bglS* mutants contained pAR18 (Table 2). The complementation to Arb$^+$ by pMN5 indicates that the *bglS* gene is contained within the 2.3-kb *Hin*dIII-*Cla*I fragment.

The analyses described above indicate the presence of three *bgl* structural genes located downstream of *bglR* in the order *bglC bglS bglB*.

Identification of the regulatory genes. Analysis of *bgl* operon regulation was carried out by using a *bgl-lacZ* transcriptional fusion constructed as described in Materials and Methods. The fusion, present in single copy on a λ prophage, contained the *bgl* promoter including the activating IS*5* mutation and a portion of the *bglC* gene. Regulation of expression of the fusion was studied by measuring β-galactosidase activity in strains with different *bgl* genotypes (Table 3). In strain MA152, which is deleted for the chromosomal *bgl* operon, little expression from the *bgl-lacZ* fusion was detected by colony color on MacConkey lactose plates or by β-galactosidase assays. However, constitutive expression of the fusion was observed when plasmid pMN25, carrying the complete *bglC* gene, was present in the cell. Plasmid pMN5, which expresses only the *bglS* gene, had no effect on the expression of the fusion. In the presence of plasmid pAR18 (*bglC*$^+$ *bglS*$^+$) or plasmid pSAL6 (*bglC*$^+$ *bglS*$^+$ *bglB*$^+$), the expression of the fusion was inducible by β-glucosides. The same result was observed in the Bgl$^+$ strain MA200. These observations indicate that the *bglC* gene product is absolutely required for the expression of the *bgl-lacZ* fusion and, in the absence of the *bglS* gene, leads to constitutive expression of the fusion. Therefore, *bglC* specifies a positive regulator of expression of the *bgl-lacZ* fusion. When the *bglC* and *bglS* genes are simultaneously present, expression of the *bgl-lacZ* fusion requires a β-glucoside inducer, indicating a negative role for the *bglS* gene product in regulating *bgl* expression.

Mutations leading to constitutive expression of the *bgl-lacZ* fusion. Regulation of *bgl* operon expression was further characterized by selecting for mutations that result in con-

TABLE 2. Complementation analysis of Bgl$^-$ mutants[a]

Strain	Complementation with plasmids:				
	pAR10	pMN25	pAR18	p1H	p6J
AE304-1	−	−	−	+	−
AE304-2	−	−	−	+	−
AE304-3	−	−	−	+	+
AE304-4	−	−	−	+	−
AE304-6	−	−	−	+	+
AE304-7	±	+	+	−	−
AE304-9	±	+	+	−	−
AE304-10	−	±	±	−	−
MA221	±	+	+	−	−
MA222	−	±	±	−	−
MA223	−	−	−	+	+
MA225	−	−	−	+	+
MA226	−	−	−	+	+
MA227	−	−	−	+	+
MA229	−	−	−	+	+
MA231	−	−	−	+	−
MA233	±	+	+	−	−
MA234	−	−	−	+	+

[a] Complementation was indicated by colony color on MacConkey salicin plates: (+) strong complementation (bright red colonies), (±) partial complementation (pale pink colonies), (−) no complementation (white colonies).

TABLE 3. Expression of a *bgl-lacZ* transcriptional fusion in various strain backgrounds

Strain	Plasmid	Phenotype on MacConkey-lactose medium[a]		β-Galactosidase activity (U)	
		−Inducer	+Inducer[b]	−Inducer	+Inducer[c]
MA110 (control)	None	−	−	<1	<1
MA152 (Δ*bgl*)	None	−	−	4	3
MA152	pMN25 (*bglC*$^+$)	+	+	54	58
MA152	pMN5 (*bglS*$^+$)	−	−	ND[d]	ND
MA152	p6J (*bglB*$^+$)	−	−	ND	ND
MA152	pAR18 (*bglC*$^+$ *bglS*$^+$)	−	+	ND	ND
MA152	pSAL6 (Bgl$^+$)	−	+	4	48
MA200 (Bgl$^+$)	None	−	+	3	43

[a] Expression of the *bgl-lacZ* fusion was partly determined by colony color on MacConkey lactose plates: (+) pink colonies, (−) white colonies.
[b] β-Methylglucoside (10 mM) was used as the inducer.
[c] Salicin (7 mM) was used as the inducer.
[d] ND, Not determined.

stitutive expression of the *bgl-lacZ* fusion. Strain MA200, a Bgl$^+$ strain lysogenic for the λ phage carrying the transcriptional fusion, was used to isolate mutants that express the fusion constitutively. Spontaneous Lac$^+$ mutants were isolated by their ability to grow on minimal lactose medium in the absence of a β-glucoside inducer. The major class of spontaneous Lac$^+$ derivatives (83 of 84) had a Lac^c Bgl$^-$ phenotype. Four representative strains from this class, MA200-1 through -4, were chosen for detailed analysis. A single Lac^c isolate, MA200-33, showed constitutive expression of the *bgl* operon (see below).

The observation that the Lac^c mutations present in strains MA200-1, through -4 had simultaneously acquired a Bgl$^-$ phenotype suggested that the mutations were likely to map within the chromosomal *bgl* operon. To determine whether the mutations were linked to the chromosomal *bgl* operon, *rec*$^+$ derivatives of the mutant strains were transduced with P1 phages grown on a Bgl$^+$ strain carrying a Tn*10* insertion in *tna* linked to the *bgl* operon. Tet^r transductants were screened for Bgl and Lac phenotypes. About 80% of the Tet^r transductants showed a Bgl$^+$ phenotype. Transduction to Bgl$^+$ simultaneously resulted in inducible expression of the *bgl-lacZ* fusion, similar to the original strain MA200. In addition, λ phages carrying the *bgl-lacZ* fusion were isolated from the four Lac^c mutants and used to relysogenize the Bgl$^+$ strain MA10. These lysogens showed inducible expression of the *bgl-lacZ* fusion, similar to the original lysogen MA200, indicating that the λ prophages carrying the fusion, present in MA200-1 through -4, are unaltered. Therefore, the mutations leading to constitutive expression of the fusion and simultaneous loss of *bgl* expression are linked to the chromosomal *bgl* operon.

Mutations in MA200-1 through -4 were mapped more precisely by complementation analysis with the deletion plasmids described above (Table 4). Plasmids pMN25 and p6J, expressing the *bglC* and *bglB* genes, respectively, had no effect on the strains. However, transformants of the mutant strains carrying plasmid p1H, which expresses the *bglS* and *bglB* genes, showed an Arb$^+$ Sal$^+$ phenotype. The presence of an intact *bglS* gene also resulted in inducible expression of the *bgl-lacZ* fusion. These results indicate that the mutations in strains MA200-1 through -4, which lead to constitutive expression of the *bgl-lacZ* fusion and simultaneously result in a Bgl$^-$ phenotype, are recessive and are complemented by a plasmid expressing the *bglS* gene. Hence, we conclude that these mutations map within the *bglS* gene. This is consistent with our observation that a derivative of strain MA231 (*bglS*), which carries the *bgl-lacZ* fusion, shows constitutive expression of the fusion. The presence of a plasmid expressing the wild-type *bglS* gene in this strain also results in inducible expression of the fusion. These results indicate once again a negative role for the *bglS* gene product in regulating the expression of the *bgl-lacZ* fusion.

Plasmid pMN5, which expresses only the *bglS* gene, complemented the mutations described above, resulting in an Arb$^+$ phenotype. However, these strains remained Sal$^-$, suggesting that the mutations in strains MA200-1 through -4, are also polar on the *bglB* gene, similar to the independently isolated *bglS* mutants described earlier (Table 2).

TABLE 4. Properties of mutants which show constitutive expression of the *bgl-lacZ* fusion[a]

Strain	Plasmid	Phenotype on MacConkey medium plus:			β-Galactosidase activity (U)	
		Salicin[b]	Lactose[c]		−Inducer	+Inducer[d]
			−Inducer	+Inducer[e]		
MA200 (Bgl$^+$)	None	+	−	+	3	43
MA200-1	None	−	+	+	194	176
MA200-1	pMN25 (*bglC*$^+$)	−	+	+	ND[f]	ND
MA200-1	p6J (*bglB*$^+$)	−	+	+	ND	ND
MA200-1	p1H (*bglS*$^+$ *bglB*$^+$)	+	−	+	ND	ND
MA200-1	pMN5 (*bglS*$^+$)	[−]	−	+	3	41

[a] Data for one of the four Lac^c mutants characterized is shown. Three other mutants tested showed similar complementation pattern and β-galactosidase levels.
[b] Representation of phenotype on MacConkey salicin plates is as in Table 2. Complementation that resulted in a Sal$^-$ phenotype on MacConkey salicin plate but an Arb$^+$ phenotype on MacConkey arbutin plates is indicated as [−].
[c] Phenotypes on MacConkey lactose plates are represented as in Table 3.
[d] Salicin (7 mM) was used as the inducer.
[e] β-Methylglucoside (10 mM) was used as the inducer.
[f] ND, Not determined.

The levels of expression of the *bgl-lacZ* fusion in *bglS* mutants are higher compared to the induced levels of expression in Bgl$^+$ strains (Table 4). This difference is likely to be related to catabolite repression caused by the production of glucose 6-phosphate from salicin (the inducer) in the Bgl$^+$ strain. Since the *bglS* mutants show a Bgl$^-$ phenotype and are unable to utilize the inducer as a substrate, no catabolite repression is likely to occur in these strains.

Mutations leading to constitutive *bgl* expression. In their original studies on *bgl* operon expression, Prasad and Schaefler described a single mutation that resulted in constitutive expression of the *bgl* operon (7). Initial mapping indicated that the mutation was located near the *bglB* locus. With merodiploid analysis, the mutant allele was shown to be dominant in *trans* over the wild type. Based on this observation, Prasad and Schaefler proposed that the mutation defines a gene which encodes a positive regulator of *bgl* operon expression.

We analyzed the mutation described by Prasad and Schaefler after transferring it from the original strain SP3 to a *bgl* plasmid by P1 transduction. The Bgl$^+$ plasmids obtained, pAR16 and pAR17, were found to be constitutive for *bgl* expression, indicating that the mutation in SP3 had been successfully transferred to the plasmids. To map the mutation, a series of hybrid plasmids was constructed by ligating specific DNA fragments from plasmids pAR16 (Bgl^c) and pSAL6 (Bgl$^+$). The properties of recombinant plasmids (Fig. 4) indicated that the mutation present in strain SP3, which confers constitutive expression of the *bgl* operon, lies in the 0.2-kb region (between the *Hin*dIII and *Hpa*I sites) within the gene that we have designated *bglC*. This is consistent with our observation that the *bglC* gene encodes a positive regulator of *bgl* operon expression. We have designated the *bglC* allele, present in strain SP3, *bglC4*.

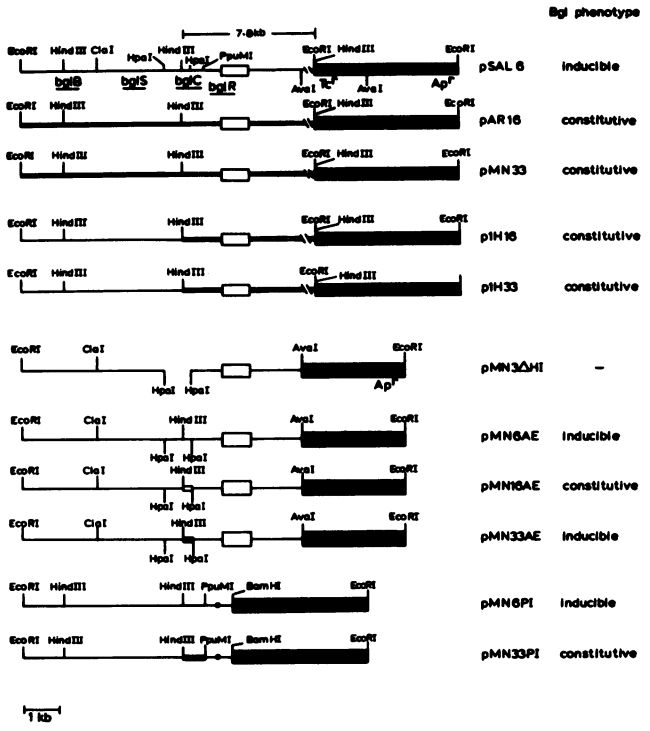

FIG. 4. Mapping of mutations leading to constitutive expression of the *bgl* operon. Symbols: □, activating IS*1* insertions; ■, pBR322 sequences; ———, DNA from plasmid pSAL6 (*bglC*$^+$); ═, DNA from plasmid pAR16 (*bglC4*); ▬, DNA from plasmid pMN33 (*bglC33*). The *Ppu*MI-*Bam*HI restriction fragment containing the *bglR* region present in plasmids pMN6PI and pMN33PI was derived from plasmid pMN25. The activating mutation present in these two cases is a point mutation in *bglR* indicated (●). The phenotype indicated was observed in a Δ*bgl* strain. Constitutive expression was detected as described in Materials and Methods.

TABLE 5. Effects of multiple copies of *bglC* and *bglS* genes on regulation

Strain	Plasmid	β-Galactosidase activity (U)	
		−Inducer	+Inducer[a]
MA200 (Bgl$^+$)	pBR322	3	40
MA200	pMN25 (*bglC*$^+$)	5	47
MA200	pMN5 (*bglS*$^+$)	3	15
MA200	pSAL6 (Bgl$^+$)	4	49
MA46-200 (*bglC4*)	pBR322	46	110
MA46-200	pMN25 (*bglC*$^+$)	39	227
MA46-200	pMN5 (*bglS*$^+$)	10	16
MA200-33 (*bglC33*)	pBR322	160	57
MA200-33	pMN25 (*bglC*$^+$)	64	33
MA200-33	pMN5 (*bglS*$^+$)	123	33

[a] Salicin (7 mM) was used as the inducer.

A second mutation exhibiting constitutive *bgl* expression, present in strain MA200-33, was obtained by screening the spontaneous Lac$^+$ constitutive mutants of MA200 described in the previous section. Among the 84 Lac^c derivatives screened, one (MA200-33) showed constitutive *bgl* expression. Strain MA200-33 showed constitutive *bgl* expression even when it contained the Bgl$^+$ plasmid pSAL6, indicating that the mutation in strain MA200-33 is dominant. When P1 phages grown on a Bgl$^+$ strain carrying a Tn*10* insertion in *tna* were used to transduce a *recA*$^+$ derivative of MA200-33, 80% of the Tet^r transductants showed inducible *bgl* expression. This result indicated that the mutation in strain MA200-33 is linked to the chromosomal *bgl* operon.

To localize the mutation in strain MA200-33 more precisely, the mutation was transferred to a plasmid by P1 transduction as in the previous case. The resulting plasmid, pMN33, gave constitutive expression of the *bgl* operon. The properties of hybrid plasmids, constructed with DNA fragments from pMN33 and pSAL6 (Fig. 4), indicated that the mutation in strain MA200-33, which leads to constitutive expression of the *bgl* operon and the *bgl-lacZ* fusion, is located within the 0.4-kb region upstream of the *Hpa*I restriction site in *bglC*, i.e., within the *bglC* structural gene. This location of the *bglC33* allele indicates that it is distinct from the *bglC4* allele.

Effect of increased dosage of the *bglC* and *bglS* genes on regulation. To understand the mode of action of the regulatory genes, the effect of *bglC* and *bglS* in high copy on *bgl* regulation was studied in strains containing the *bgl-lacZ* fusion and a single copy of the chromosomal *bgl* operon (Table 5). The *bglC*$^+$ plasmid pMN25 had no effect on the expression of the *bgl-lacZ* fusion in the Bgl$^+$ strain MA200, which carries a single copy of the *bglC* and *bglS* genes on the chromosome. However, when the *bglS*$^+$ plasmid pMN5 was present in strain MA200, the induced level of β-galactosidase was reduced over twofold. In the presence of the Bgl$^+$ plasmid pSAL6 (*bglC*$^+$ *bglS*$^+$), the expression of the *bgl-lacZ* fusion in MA200 was unaltered. Thus, the presence of multiple copies of the *bglS* gene in *trans* to a single copy of the *bglC* gene has a negative effect on the expression of the *bgl-lacZ* fusion.

The effect of higher copy numbers of *bglC* and *bglS* on the expression of the *bgl-lacZ* fusion was studied with single copies of the *bglC4* and *bglC33* alleles on the chromosome. Strains MA46-200 (*bglC4*) and MA200-33 (*bglC33*) showed partially constitutive expression of the *bgl-lacZ* fusion in the presence of the $bglC^+$ plasmid pMN25, indicating the *trans*-dominant phenotype of the two *bglC* alleles. The $bglS^+$ plasmid, pMN5, caused a 75% reduction in the uninduced levels of expression of the *bgl-lacZ* fusion in strain MA46-200. Thus, the presence of a multicopy plasmid expressing *bglS* suppressed the constitutive phenotype of the *bglC4* allele. However, in contrast to the result obtained with the *bglC4* allele, the presence of the *bglC33* allele led to constitutive expression of the *bgl-lacZ* fusion even when plasmid pMN5 ($bglS^+$) was present. Over 75% of the original uninduced level of β-galactosidase activity of MA200-33 could be detected in the presence of pMN5. Multiple copies of the *bglS* gene are unable to effectively suppress the constitutive phenotype of the *bglC33* allele. This result suggests that the opposing effects exerted by the *bglS* and the *bglC* gene products are not independent.

DISCUSSION

Organization of the *bgl* genes. We have shown that the *bgl* structural genes, which are required for regulated *bgl* expression, are encoded by the 5.7 kb of DNA located downstream of the *bglR* site. Since the *bglR* site has been shown to contain the promoter elements involved in *bgl* expression (10), our results indicate that the *bgl* structural genes must be transcribed unidirectionally from *bglR* rather than bidirectionally as proposed earlier (7). This is also confirmed by the nucleotide sequence analysis of the *phoSTU* genes immediately upstream of *bglR* (16). The *phoU* open reading frame has been shown to end just upstream of the target region for the insertion sequences in *bglR*. Therefore, it is not likely that the region upstream of *bglR* encodes a *bgl* structural gene. Hence, we conclude that the *bgl* structural genes are contained within the 5.7-kb region downstream of the *bglR* site and are transcribed unidirectionally from *bglR*, constituting an operon.

The results of our analysis of the various Bgl^- mutants have indicated that there are at least three structural genes in the operon. We have designated the first structural gene of the operon *bglC* and the second gene *bglS*. The *bglB* gene encoding the enzyme phospho-β-glucosidase B, which mapped downstream of *bglS*, constitutes the third structural gene of the operon.

Rak and co-workers have determined the nucleotide sequence of the *bgl* operon, and their results were communicated to us while the manuscript of this paper was in preparation. Their analysis has confirmed the presence of three open reading frames bounded by the same restriction sites defined by our genetic analysis. The conclusions based on the nucleotide sequence analysis of the operon are presented in the accompanying paper by Schnetz et al. (12).

Role of *bglC* and *bglS* in the regulation of the *bgl* operon. Our studies with a *bgl-lacZ* transcriptional fusion have shown that the presence of *bglC* in *trans* is necessary and sufficient for the expression of the fusion in strains deleted for the chromosomal *bgl* operon. Since the expression of the fusion does not require a β-glucoside inducer in this case, it is unlikely that the requirement for the *bglC* gene product is related to an indirect process, such as uptake of β-glucosides. We have also shown that loss of *bglC* function due to mutations results in a Bgl^- phenotype, whereas rare mutations in *bglC* result in constitutive expression of the operon. Hence, we conclude that the *bglC* gene specifies a positive regulator of *bgl* operon expression.

The studies with the *bgl-lacZ* transcriptional fusion have also shown that the *bglS* gene product is a negative regulator of *bgl* operon expression. In the presence of *bglS* and *bglC*, the expression of the fusion requires a β-glucoside inducer. Mutations that inactivate the *bglS* gene result in constitutive expression of the fusion. The single copy of the chromosomal *bglC* gene, in this case, stimulates the expression of the *bgl-lacZ* fusion in the absence of a β-glucoside inducer (Table 4). Therefore, induction of the operon is not likely to occur through the direct activation of the *bglC* gene product by the inducer. We propose that β-glucosides induce the *bgl* operon by relieving the negative effect exerted by the *bglS* gene product.

The dual regulation of the *bgl* operon leads to two possible schemes for the operation of the regulatory components. The *bglC* and *bglS* gene products could exert their opposing effects either independently or concurrently at the same level of transcriptional regulation. The phenotype of the two *bglC* mutations (*bglC4* and *bglC33*) that lead to constitutive expression of the *bgl* operon indicates that a mutation in the *bglC* gene, in a single step, can overcome the negative effect of the *bglS* gene product. Therefore, it is more likely that the *bglC* and *bglS* gene products act at the same level of regulation, either by competing for a common regulatory site or by directly interacting with each other. The *bglC4* and *bglC33* alleles are not likely to be mutations that increase the steady-state level of the *bglC* gene product, since increasing the copy number of the wild-type *bglC* gene in a cell carrying a single copy of the *bglS* gene has no appreciable effect on the expression of the *bgl-lacZ* fusion (Table 5). In addition, the presence of the wild-type *bglS* gene in multiple copies does not seem to have an appreciable effect on the phenotype of the *bglC33* allele, as indicated by the measurements of the expression of the *bgl-lacZ* fusion. The phenotype of the *bglC4* allele, however, has been shown to be suppressed under the same conditions. Hence, it is most likely that the *bglS* gene product exerts its negative effect by directly interfering with the efficient functioning of the *bglC* gene product. The *bglC4* and *bglC33* mutations are apparently overcoming the inhibition by *bglS*, the *bglC4* allele being weaker than *bglC33* in this respect. We hypothesize that induction of the *bgl* operon by β-glucosides occurs as a result of a structural alteration of the *bglS* gene product in the presence of the inducer, which preempts its inhibitory effect on the *bglC* gene product.

Role of *bglS* in β-glucoside utilization. All of the *bglS* mutants characterized in this study exhibit a Bgl^- phenotype. The presence of the plasmid pMN5, expressing the *bglS* gene, did not restore the Sal^+ phenotype in these strains, indicating that the *bglS* mutants are polar on the *bglB* gene. The *bglS* mutants retained their Sal^- phenotype even in the presence of plasmid p6J expressing the *bglB* gene. Since this strain is still incapable of salicin utilization despite the expression of phospho-β-glucosidase B, the *bglS* gene product must be required in some capacity other than as a negative regulator. The presence of the plasmid pMN5 could restore the Arb^+ phenotype in these mutants, indicating that the *bglS* gene product could facilitate utilization of arbutin. Based on these observations, we propose that the *bglS* gene product is the *bgl*-specific component of the phosphotransferase system that is required for the uptake of β-glucosides.

Does the *bglC* gene product play a direct role in β-

glucoside utilization other than as a positive regulator of *bgl* operon expression? The observation that plasmid p1H, expressing the *bglS* and *bglB* genes from a vector promoter, still exhibits an Arb^- Sal^- phenotype in *bglC* mutants (Table 2) suggested that the *bglC* gene product may also be involved in the uptake of β-glucosides. However, plasmid derivatives that carry deletions of the *bglC* gene, extending toward the *bglS* gene, conferred an Arb^+ Sal^+ phenotype in the absence of a functional *bglC* gene. This result suggests that there may be *bglC*-dependent regulatory sites located on plasmid p1H and that deletion of these sites abolishes the requirement of the *bglC* gene product as a positive regulator. Therefore, it is not likely that the *bglC* gene product is directly involved in β-glucoside utilization other than as a positive regulator of *bgl* operon expression. Thus the *bglS* and *bglB* genes alone specify the functions necessary for the utilization of β-glucosides.

The *bgl* operon is rather unique in specifying a transport protein which also functions as a regulator of gene expression. Studies on the *put* operon of *S. typhimurium*, involved in the catabolism of proline, have indicated that the *putA* gene product is both a negative regulator of *put* operon expression and a membrane-bound bifunctional oxidase-dehydrogenase (4). The *glnA* gene product of *B. subtilis*, which constitutes the enzyme glutamine synthetase, has been implicated in the autoregulation of the *glnA* gene (13). Hence the presence of regulatory components with multiple functions may be a more common feature among procaryotes than currently understood.

Mode of action of the *bglC* gene product. Several possible schemes can be postulated for the role of the *bglC* gene product in transcriptional control. The three basic possibilities are that the *bglC* gene product functions at the level of (i) transcription initiation, (ii) stabilization of the *bgl* transcript, or (iii) antitermination at a specific site downstream of the *bgl* promoter. We have several lines of evidence which indicate that the *bglC* gene product mediates positive regulation of the *bgl* operon at the level of antitermination of transcription at a specific *rho*-independent terminator, downstream of the transcription start site. These results will be reported elsewhere.

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