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

Received 20 November 1986/Accepted 5 March 1987

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 ISI or IS5 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 regulator 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
<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE10</td>
<td>F− ΔlacX74 thi bglR11 (bglR::IS1) (Bgl+)</td>
<td>Felton</td>
</tr>
<tr>
<td>AE304</td>
<td>As AE10 and tna: Tnl0 bglR bglSI</td>
<td></td>
</tr>
<tr>
<td>AE304-1</td>
<td>As AE304 and tna: Tnl0 bglR bglSI</td>
<td></td>
</tr>
<tr>
<td>AE304-2</td>
<td>As AE304 and tna: Tnl0 bglR bglS2</td>
<td></td>
</tr>
<tr>
<td>AE304-3</td>
<td>As AE304 and tna: Tnl0 bglR bglB3(Am)</td>
<td></td>
</tr>
<tr>
<td>AE304-4</td>
<td>As AE304 and tna: Tnl0 bglR bglS3</td>
<td></td>
</tr>
<tr>
<td>AE304-6</td>
<td>As AE304 and tna: Tnl0 bglR bglB6</td>
<td></td>
</tr>
<tr>
<td>AE304-7</td>
<td>As AE304 and tna: Tnl0 bglR bglC7(Am)</td>
<td></td>
</tr>
<tr>
<td>AE304-9</td>
<td>As AE304 and tna: Tnl0 bglR bglC8</td>
<td></td>
</tr>
<tr>
<td>AE304-10</td>
<td>As AE304 and tna: Tnl0 bglR bglC10</td>
<td></td>
</tr>
<tr>
<td>AE325</td>
<td>F− bglR trpB procC:: Tnl5 invo tna-5 (Bgl+)</td>
<td></td>
</tr>
<tr>
<td>JF50</td>
<td>F− Δlac mel gyrA supF</td>
<td>J. Felton</td>
</tr>
<tr>
<td>JF201</td>
<td>F− ΔlacX74 Δ(glucose-pho)201 ara thi gyrA</td>
<td></td>
</tr>
<tr>
<td>MA10</td>
<td>As AE10 and srl: Tnl0 recA56 (Bgl+)</td>
<td></td>
</tr>
<tr>
<td>MA46</td>
<td>As MA10 and bglR::IS1 bglB:: Tnl5 bglC4</td>
<td></td>
</tr>
<tr>
<td>MA46-200</td>
<td>As MA46 and λ bglR7 bglC− lacZ+ lacY+ (Bgl−)</td>
<td></td>
</tr>
<tr>
<td>MA110</td>
<td>As AE10 tna and Δ(glucose-pho) srl:: Tnl0 recA56</td>
<td></td>
</tr>
<tr>
<td>MA152</td>
<td>As MA110 and λ bglR7 bglC− lacZ+ lacY+ (Bgl−)</td>
<td></td>
</tr>
<tr>
<td>MA200</td>
<td>As MA110 and λ bglR7 bglC− lacZ+ lacY+ (Bgl−)</td>
<td></td>
</tr>
<tr>
<td>MA200-1</td>
<td>As MA200 and bglS201</td>
<td></td>
</tr>
<tr>
<td>MA200-2</td>
<td>As MA200 and bglS202</td>
<td></td>
</tr>
<tr>
<td>MA200-3</td>
<td>As MA200 and bglS203</td>
<td></td>
</tr>
<tr>
<td>MA200-4</td>
<td>As MA200 and bglS204</td>
<td></td>
</tr>
<tr>
<td>MA200-33</td>
<td>As MA200 and bglC33 (Bgl+)</td>
<td></td>
</tr>
<tr>
<td>MA221</td>
<td>As AE325 and tna− recA56 lac bgIC21</td>
<td></td>
</tr>
<tr>
<td>MA222</td>
<td>As AE325 and tna− recA56 lac bgIC22</td>
<td></td>
</tr>
<tr>
<td>MA223</td>
<td>As AE325 and tna− recA56 lac bgIC23(Ts)</td>
<td></td>
</tr>
<tr>
<td>MA225</td>
<td>As AE325 and tna− recA56 lac bgIC25</td>
<td></td>
</tr>
<tr>
<td>MA226</td>
<td>As AE325 and tna− recA56 lac bgIC26(Ts)</td>
<td></td>
</tr>
<tr>
<td>MA227</td>
<td>As AE325 and tna− recA56 lac bgIC27</td>
<td></td>
</tr>
<tr>
<td>MA229</td>
<td>As AE325 and tna− recA56 lac bgIC29</td>
<td></td>
</tr>
<tr>
<td>MA231</td>
<td>As AE325 and tna− recA56 lac bgIC31</td>
<td></td>
</tr>
<tr>
<td>MA233</td>
<td>As AE325 and tna− recA56 lac bgIC33</td>
<td></td>
</tr>
<tr>
<td>MA234</td>
<td>As AE325 and tna− recA56 lac bgIC34</td>
<td></td>
</tr>
<tr>
<td>SP3</td>
<td>F− bglR1 (bglR::IS1) bglC4 lamB (Bgl+)</td>
<td>Peters</td>
</tr>
<tr>
<td>WP72</td>
<td>F− galE arg pro thi rpsL tna::Tnl0 bglR (Bgl+)</td>
<td></td>
</tr>
</tbody>
</table>

**Regulation of the bgl Operon in E. coli**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANF1955</td>
<td>λ cI857 λ Sam100 lacZ' lacY' 14</td>
<td></td>
</tr>
<tr>
<td>AMN200</td>
<td>λ c− S+ bglR7 bglC− lacZ' lacY' Φ(bgl-lac)</td>
<td></td>
</tr>
</tbody>
</table>

**Plasmids**

- pAR6: bglR6 bglC+ bglS+ bglB+ (Bgl−) glmS+ phoS+ phoT+ phoU+ Ap' Tcr' 10
- pAR7: As pAR6 and bglR7 (bglR::IS5) (Bgl−)
- pAR8: As pAR7 Δ(glm-pho-IS5') (Bgl−)
- pAR10: bglR7 bglC+ Ap' 3
- pAR16: bglR1 bglC4 bglS+ bglB+ Ap' Tc' (Bgl+)
- pAR18: bglR7 bglC+ bglS+ bglB+ Ap' 3
- pMB041: trpA+-lacZ lacY+ lacA' Apr 3
- pMN5: bglC+ bglS+ bglB+ Apr 3
- pSAL6: bglR3 (bglR::IS1) bglC+ bglS+ bglB+ Ap' Tc' (Bgl+)
- p1H: bglC+ bglS' bglB+
- p61: bglS' bglB+

*Strains, plasmids, 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 IS1 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 Tnl0 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 Tnl0 insertion in srl after transducing the tna::Tnl0 mutation to tna+.

The strain JF201, which carries a deletion of the chromosomal bgl operon and the adjacent phoUT genes, was derived previously (10). The phoUT deletion results in the constitutive synthesis of alkaline phosphatase encoded by the phoA gene. The Pho6 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::Tnl0 marker. Tet+ transductants were screened for a Bgl− Pho6 phenotype. The recA56 allele was introduced after selecting for Tet+ clones (1). Strain MA46 was constructed by transducing the bglC4 allele from strain SP3 with a Tn5 insertion in bglB. The presence of the bglC4 allele was confirmed by screening Kan− transductants for an Arb+ 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 IS5 insertion in bglR. Plasmids pAR10 and pAR18 were generated by deleting sequences from pAR7 with HindIII restriction sites. The bglC+ plasmid pMN25 was constructed by subcloning the 0.5-kb HindIII-HpaI restriction fragment from pSAL6.
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 Tn10 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" 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" transductants were used for further analysis. Mutations in the bglIB gene were identified by their characteristic Ar" 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 IS5-activated bgl promoter and a portion of the blgC gene to lacZ. This construction strategy is outlined in Fig. 1. A 12-kb HindIII-BamHI fragment containing the bgl sequences and the pBR322 vector, derived from plasmid pAR7, was isolated and ligated with an 8-kb HindIII-BamHI fragment containing the lacZYA genes derived from the plasmid pMBO41 (3). The ligated DNA was used to transform the Bgl" strain MA10. Amp" 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 EcoRI 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

![Diagram of bgl-lacZ transcriptional fusion](image)

**FIG. 1.** Construction of a bgl-lacZ transcriptional fusion. Symbols: □, activating IS5 insertion in bglR; ■, pBR322 sequences; ▲ λ sequences. Plasmid pMBO41 is a derivative of pMBO40 (3) in which a 205-base-pair EcoRI restriction fragment carrying the lacUV5 promoter has been deleted.

downstream of the unique HindIII site in plasmid pAW25 (10). This construction reconstitutes the bgl sequences from the bglIR site to the second HpaI site in the operon (see Fig. 3).

The plasmid p1H was constructed by partial digestion of pSAL6 DNA with HindIII and subsequent ligation. The bglIS" plasmid pMN5 was derived by subcloning a 2.3-kb HindIII-ClaI restriction fragment from pSAL6 into the HindIII-ClaI site of pBR322. HpaI 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 recipes (6). Media used for growing strains carrying pBR322-derived plasmids contained 100 μg
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. EcoRI restriction fragments from the Bgl + plasmid pAR7, which contains an EcoRI site in the activating IS5 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 IS5 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 EcoRI 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 HindIII 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 HindIII site but does not extend beyond the second HpaI 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 HindIII site and upstream of the second HpaI 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
from the activity carrying

The been omitted. Symbols: \(\square\), activating ISS insertion in bgIR; \(\bullet\), point mutation in bgIR; \(\copyright\), transcription from the bgIR promoter; \(\circ\), transcription from the pBR322 vector.

![Diagram](image-url)

**Fig. 3.** Structural organization of the bgIR 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: \(\square\), activating ISS insertion in bgIR; \(\bullet\), point mutation in bgIR; \(\copyright\), transcription from the bgIR promoter; \(\circ\), transcription from the pBR322 vector.

Structural gene encoding the enzyme phospho-\(\beta\)-glucosidase B, which preferentially cleaves phosphosalicin (7). Strains with bgIB mutations can utilize arbutin, by using the unlinked locus bgLA (8), and the \(\beta\)-glucoside transport system I, specified by the bgIR operon (7). Plasmid pAR18, which carries bgIR DNA up to the second HindIII site in the operon (Fig. 3), confers an Arb\(^+\) Sal\(^-\) phenotype to cells deleted for the chromosomal bgIR operon, indicating that this HindIII site lies within the bgIR gene.

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

The analyses described above indicate the presence of three bgI structural genes located downstream of bgIR in the order bgIC bgIS bgIB.

**Identification of the regulatory genes.** Analysis of bgIR operon regulation was carried out by using a bgIR-lacZ transcriptional fusion constructed as described in Materials and Methods. The fusion, present in single copy on a \(\lambda\) prophage, contained the bgIR promoter including the activating ISS mutation and a portion of the bgIC gene. Regulation of expression of the fusion was studied by measuring \(\beta\)-galactosidase activity in strains with different bgI genotypes (Table 3). In strain MA152, which is deleted for the chromosomal bgIR operon, little expression from the bgIR-lacZ fusion was detected by colony color on MacConkey lactose plates or by \(\beta\)-galactosidase assays. However, constitutive expression of the fusion was observed when plasmid pMN25, carrying the complete bgIC gene, was present in the cell. Plasmid pMN5, which expresses only the bgIS gene, had no effect on the expression of the fusion. In the presence of plasmid pAR18 (bgIC\(^+\) bgIS\(^+\)) or plasmid pSA6 (bgIC\(^+\) bgIS\(^+\) bgIB\(^+\)), the expression of the fusion was inducible by \(\beta\)-glucosides. The same result was observed in the Bgl\(^+\) strain MA200. These observations indicate that the bgIC gene product is absolutely required for the expression of the bgIR-lacZ fusion and, in the absence of the bgIS gene, leads to constitutive expression of the fusion. Therefore, bgIC specifies a positive regulator of expression of the bgIR-lacZ fusion. When the bgIC and bgIS genes are simultaneously present, expression of the bgIR-lacZ fusion requires a \(\beta\)-glucoside inducer, indicating a negative role for the bgIS gene product in regulating bgIR expression.

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

### Table 2. Complementation analysis of Bgl\(^-\) mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>pAR10</th>
<th>pMN25</th>
<th>pAR18</th>
<th>p1H</th>
<th>p6J</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE304-1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AE304-2</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AE304-3</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AE304-4</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AE304-6</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>AE304-7</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>AE304-9</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>AE304-10</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA221</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA222</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA223</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MA225</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MA226</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MA227</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MA228</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MA229</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>MA231</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA233</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>MA234</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

*Complementation was indicated by colony color on MacConkey salicin plates: (+) strong complementation (bright red colonies), (±) partial complementation (pale pink colonies), (−) no complementation (white colonies).*
In the absence of a b-glucoside inducer, the bgl-lacZ fusion was partly determined by colony color on MacConkey lactose plates: (+) pink colonies, (−) white colonies. b-Methylglucoside (10 mM) was used as the inducer. Salicin (7 mM) was used as the inducer. ND, Not determined.

The observation that the Lacc mutations present in strains MA200-1, through -4 had simultaneously acquired a Bglc phenotype suggested that the mutations were likely to be linked within the chromosomal bgl operon. To determine whether the mutations were linked to the chromosomal bgl operon, recc derivatives of the mutant strains were transduced with P1 phages grown on a Bglc strain carrying a Tn10 insertion in tna linked to the bgl operon. Tet + transductants were screened for Bgl and Lac phenotypes. About 80% of the Tet + transductants showed a Bglc phenotype. Transduction to Bglc 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 Lacc mutants and used to relysogenize the Bglc strain MA10. These lysogens showed inducible expression of the bgl-lacZ fusion, similar to the original lysogen MA200, indicating that the λ phages 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 2). 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 pH, 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 Bglc 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Salicin</th>
<th>Lactose&lt;sup&gt;a&lt;/sup&gt;</th>
<th>β-Galactosidase activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-Inducer</td>
<td>+Inducer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-Inducer</td>
</tr>
<tr>
<td>MA200 (Bgl&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>None</td>
<td>−</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>MA200-1</td>
<td>None</td>
<td>−</td>
<td>+</td>
<td>194</td>
</tr>
<tr>
<td>MA200-1</td>
<td>pMN25 (bgl&lt;sup&gt;C&lt;/sup&gt;)</td>
<td>−</td>
<td>+</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MA200-1</td>
<td>p6J (bgl&lt;sup&gt;B&lt;/sup&gt;)</td>
<td>−</td>
<td>+</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MA200-1</td>
<td>pH (bgl&lt;sup&gt;IS&lt;/sup&gt; bgl&lt;sup&gt;B&lt;/sup&gt;)</td>
<td>−</td>
<td>+</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MA200-1</td>
<td>pMN5 (bgl&lt;sup&gt;S&lt;/sup&gt;)</td>
<td>−</td>
<td>+</td>
<td>3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data for one of the four Lacc mutants characterized is shown. Three other mutants tested showed similar complementation pattern and β-galactosidase levels.

<sup>b</sup> Phenotypes 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 [−].

<sup>c</sup> Phenotypes on MacConkey lactose plates are represented as in Table 3.

<sup>d</sup> Salicin (7 mM) was used as the inducer.

<sup>e</sup> β-Methylglucoside (10 mM) was used as the inducer.

<sup>f</sup> 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 Schaeffer described a single mutation that resulted in constitutive expression of the bgl operon (7). Initial mapping indicated that the mutation was located near the bglIB locus. With merodiploid analysis, the mutant allele was shown to be dominant in trans over the wild type. Based on this observation, Prasad and Schaeffer proposed that the mutation defines a gene which encodes a positive regulator of bgl operon expression.

We analyzed the mutation described by Prasad and Schaeffer 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−) 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 HindIII and HpaI 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.

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+ 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 Tn10 insertion in tna were used to transduce a recA4 derivative of MA200-33, 80% of the Tet+ 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 HpaI 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 bglC4 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 \( bgIC \) and \( bgIS \) on the expression of the \( bgI-lacZ \) fusion was studied with single copies of \( bgIC4 \) and \( bgIC33 \) alleles on the chromosome. Strains MA46-200 (\( bgIC4 \)) and MA200-33 (\( bgIC33 \)) showed partially constitutive expression of the \( bgI-lacZ \) fusion in the presence of the \( bgIC^+ \) plasmid \( pMN25 \), indicating the trans-dominant phenotype of the two \( bgIC \) alleles. The \( bgIS^+ \) plasmid, \( pMN5 \), caused a 75% reduction in the uninduced levels of expression of the \( bgI-lacZ \) fusion in strain MA46-200. Thus, the presence of a multicopy plasmid expressing \( bgIS \) suppressed the constitutive phenotype of the \( bgIC4 \) allele. However, in contrast to the result obtained with the \( bgIC4 \) allele, the presence of the \( bgIC33 \) allele led to constitutive expression of the \( bgI-lacZ \) fusion even when plasmid \( pMN5 (bgIS^+) \) was present. Over 75% of the original uninduced level of \( \beta \)-galactosidase activity of MA200-33 could be detected in the presence of \( pMN5 \). Multiple copies of the \( bgIS \) gene are unable to effectively suppress the constitutive phenotype of the \( bgIC33 \) allele. This result suggests that the opposing effects exerted by the \( bgIS \) and the \( bgIC \) gene products are not independent.

**DISCUSSION**

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

Rak and co-workers have determined the nucleotide sequence of the \( bgI \) 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 \( bgIC \) and \( bgIS \) in the regulation of the \( bgI \) operon.** Our studies with a \( bgI-lacZ \) transcriptional fusion have shown that the presence of \( bgIC \) in \textit{trans} is necessary and sufficient for the expression of the fusion in strains deleted for the chromosomal \( bgI \) operon. Since the expression of the fusion does not require a \( \beta \)-glucoside inducer in this case, it is unlikely that the requirement for the \( bgIC \) gene product is related to an indirect process, such as uptake of \( \beta \)-glucosides. We have also shown that loss of \( bgIC \) function due to mutations results in a \( Bgl^- \) phenotype, whereas rare mutations in \( bgIC \) result in constitutive expression of the operon. Hence, we conclude that the \( bgIC \) gene specifies a positive regulator of \( bgI \) operon expression.

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

The dual regulation of the \( bgI \) operon leads to two possible schemes for the operation of the regulatory components. The \( bgIC \) and \( bgIS \) gene products could exert their opposing effects either independently or concurrently at the same level of transcriptional regulation. The phenotype of the two \( bgIS \) mutations (\( bgIC4 \) and \( bgIC33 \)) that lead to constitutive expression of the \( bgI \) operon indicates that a mutation in the \( bgIC \) gene, in a single step, can overcome the negative effect of the \( bgIS \) gene product. Therefore, it is more likely that the \( bgIC \) and \( bgIS \) 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 \( bgIC4 \) and \( bgIC33 \) alleles are not likely to be mutations that increase the steady-state level of the \( bgIC \) gene product, since increasing the copy number of the wild-type \( bgIC \) gene in a cell carrying a single copy of the \( bgIS \) gene has no appreciable effect on the expression of the \( bgI-lacZ \) fusion (Table 5). In addition, the presence of the wild-type \( bgIS \) gene in multiple copies does not seem to have an appreciable effect on the phenotype of the \( bgIC33 \) allele, as indicated by the measurements of the expression of the \( bgI-lacZ \) fusion. The phenotype of the \( bgIC4 \) allele, however, has been shown to be suppressed under the same conditions. Hence, it is most likely that the \( bgIS \) gene product exerts its negative effect by directly interfering with the efficient functioning of the \( bgIC \) gene product. The \( bgIC4 \) and \( bgIC33 \) mutations are apparently overcoming the inhibition by \( bgIS \), the \( bgIC4 \) allele being weaker than \( bgIC33 \) in this respect. We hypothesize that induction of the \( bgI \) operon by \( \beta \)-glucosides occurs as a result of a structural alteration of the \( bgIS \) gene product in the presence of the inducer, which preempts its inhibitory effect on the \( bgIC \) gene product.

**Role of \( bgIS \) in \( \beta \)-glucoside utilization.** All of the \( bgIS \) mutants characterized in this study exhibit a \( Bgl^- \) phenotype. The presence of the plasmid \( pMN5 \), expressing the \( bgIS \) gene, did not restore the \( Sal^- \) phenotype in these strains, indicating that the \( bgIS \) mutants are polar on the \( bgIB \) gene. The \( bgIS \) mutants retained their \( Sal^- \) phenotype even in the presence of plasmid \( p6J \) expressing the \( bgIB \) gene. Since this strain is still incapable of salicin utilization despite the expression of phospho-\( \beta \)-glucosidase B, the \( bgIS \) 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 \( bgIS \) gene product could facilitate utilization of arbutin. Based on these observations, we propose that the \( bgIS \) gene product is the \( bgI \)-specific component of the phospho-hexosamine transferase system that is required for the uptake of \( \beta \)-glucosides.

Does the \( bgIC \) gene product play a direct role in \( \beta \)-
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 $\beta$-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 $\beta$-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 $\beta$-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 $S.$ ryphimurium, 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.

**ACKNOWLEDGMENTS**

We thank R. Braun, Y. Fishman, J. Lopilato, and A. L. Sonenschein for discussion and helpful comments on the manuscript. Two of the plasmids used in this work were constructed by J. F. Miller.

This research was supported by grant MV96 from the American Cancer Society.

**LITERATURE CITED**


