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A Mutation in a New Gene, *bglJ*, Activates the *bgl* Operon in *Escherichia coli* K-12

Maryann Giel,[1] Martine Desnoyer[2] and Jane Lopilato

Biology Department, Simmons College, Boston, Massachusetts 02115

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ABSTRACT

A new mutation, *bglJ4*, has been characterized that results in the expression of the silent *bgl* operon. The *bgl* operon encodes proteins necessary for the transport and utilization of the aromatic β-glucosides arbutin and salicin. A variety of mutations activate the operon and result in a Bgl$^+$ phenotype. Activating mutations are located upstream of the *bgl* promoter and in genes located elsewhere on the chromosome. Mutations outside of the *bgl* operon occur in the genes encoding DNA gyrase and in the gene encoding the nucleoid associated protein H-NS. The mutation described here, *bglJ4*, has been mapped to a new locus at min 99 on the *Escherichia coli* K-12 genetic map. The putative protein encoded by the *bglJ* gene has homolgy to a family of transcriptional activators. Evidence is presented that increased expression of the *bglJ* product is needed for activation of the *bgl* operon.

β-GLUCOSIDE utilization in *Escherichia coli* K-12 requires the activation of one of several cryptic systems. Cryptic genes are phenotypically silent genes that are not normally expressed in bacteria but upon mutation can be expressed. The *bgl* operon, located at min 84 on the *E. coli* genetic map, encodes proteins necessary for the utilization of the aromatic β-glucosides salicin and arbutin (PRASAD and SCHAEFLER 1974; SCHNETZ *et al.* 1987; BACHMANN 1990). The *bgl* operon is intact but expressed at very low levels in wild-type cells; consequently, they are unable to grow on salicin. Only after mutation is the operon expressed sufficiently to result in a Bgl$^+$ phenotype. Once activated, expression of the *bgl* operon is inducible by β-glucosides (MAHADEVAN and WRIGHT 1987; MAHADEVAN *et al.* 1987; SCHNETZ and RAK 1988; AMSTER-CHODER *et al.* 1989; AMSTER-CHODER and WRIGHT 1990, 1992; HOUMAN *et al.* 1990). In addition to the *bgl* operon, there are at least three other cryptic systems for β-glucoside utilization: the *cel* operon for cellobiose utilization, the *arbT* locus for arbutin transport, and the *sac* locus for salicin-arbutin-cellobiose utilization (KRICKER and HALL 1987; PARKER and HALL 1988, 1990a,b; HALL and XU 1992). Each of these can be activated by random mutation, increasing the probability that an activated system will be present when β-glucosides are available.

Spontaneous Bgl$^+$ derivatives of *E. coli* K-12 can be isolated as papillae on indicator medium containing salicin at a frequency as high as 10^{-4} in some strains (REYNOLDS *et al.* 1981). These Bgl$^+$ mutants are due to insertion of either IS*1* or IS*5* upstream of the *bgl* promoter. The presence of these insertions most often results in activation of a preexisting but silent promoter located in the *bgl* regulatory region, *bglR* (REYNOLDS *et al.* 1985, 1986). Recently, insertions of IS*5* downstream of the *bgl* promoter have been reported that result in activation of the *bgl* promoter (SCHNETZ and RAK 1992). Activation by insertion sequences is not without drawbacks, as insertion sequence-mediated deletion of the *bgl* operon in Bgl$^+$ derivatives occurs at relatively high frequency, resulting in loss of part or all of the *bgl* operon (A. WRIGHT, unpublished observations; HALL 1988). One might therefore expect that deletion of the insertion-activated operon would lead to the eventual loss of the *bgl* operon, especially since the *cel, arbT,* and *sac* systems are available to allow the utilization of β-glucosides upon mutation. Instead, the silent *bgl* operon is present in a variety of *E. coli* strains isolated from different geographical locations (HALL 1988). The presence of such genes, which can be activated by mutation, may confer a selective advantage to those organisms that have maintained these cryptic systems (HALL *et al.* 1983). In addition, there may be some current advantage to the low level expression from the wild-type *bgl* promoter under certain environmental conditions and this may contribute to the maintenance of the *bgl* genes.

Molecular studies on the *bgl* operon have shown that the region upstream of the *bgl* promoter is a site for mutations that activate the *bgl* operon. The IS*1* and the majority of IS*5* insertions that lead to a Bgl$^+$ phenotype map to a 49-bp region upstream of the *bgl* promoter, called the target region. The target region extends from the distal edge of the CAP-cAMP binding site at postion −76 to position −125, relative to the start of transcrip-

Corresponding author: Jane Lopilato, Biology Department, Simmons College, 300 The Fenway, Boston, MA 02115.
E-mail: jlopilato@vmsvax.simmons.edu

[1] *Present address:* Oravax, 38 Sidney St., Cambridge, MA 02139.

[2] *Present address:* Biotransplant, Bldg. 75, Third Ave., Charlestown Navy Yard, Charlestown, MA 02129.

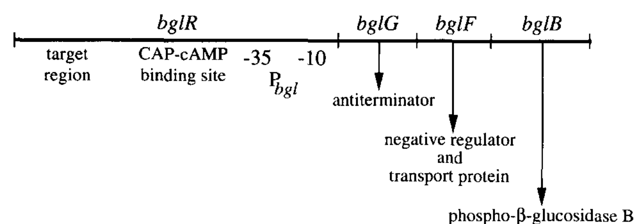

FIGURE 1.—The *bgl* operon. The regulatory region, *bglR*, of the *bgl* operon is shown that contains the target region, the CAP-cAMP-binding site, and the *bgl* promoter. The three structural genes, *bglG*, *bglF*, and *bglB*, are located downstream of a 130 nucleotide leader region (MAHADEVAN and WRIGHT 1987). The *bglG* and *bglF* genes encode regulators of transcriptional antitermination. BglF protein is also required for the transport and phosphorylation of salicin and arbutin that can then be cleaved by phospho-β-glucosidase B, the product of the *bglB* gene.

tion (Figure 1). A second class of activating mutations, isolated after mutagenesis, are due to base substitutions that improve the CAP-cAMP-binding site (REYNOLDS *et al.* 1985, 1986; LOPILATO and WRIGHT 1990). The entire region, which includes the target region, the CAP-cAMP-binding site, and the *bgl* promoter, has been designated *bglR*.

Mutations outside the *bgl* operon can also result in *bgl* expression. Certain mutations in genes encoding subunits of DNA gyrase, *gyrA* (min 48) and *gyrB* (min 83), which reduce negative supercoiling of DNA, activate the *bgl* operon (DINARDO *et al.* 1982). Mutations in the nucleoid-associated protein, H-NS, result in *bgl* expression as well. Mutations in *hns* have pleiotropic effects and formerly this gene was known as *bglY*, *cur-1*, *drdX*, *osmZ*, *pilG*, and *virR* (DEFEZ and DEFELICE 1981; PON *et al.* 1988; GORANSSON *et al.* 1990; MAY *et al.* 1990). In an *hns* mutant strain, ~36 proteins, most of which are unknown, either increase or decrease in amount (BERTIN *et al.* 1990). At least some of these proteins must be encoded by the genes regulated by *hns*. The *hns* gene itself is autoregulated and is induced by cold shock (LATEANA *et al.* 1991; FALCONI *et al.* 1993; UEGUCHI *et al.* 1993). The H-NS protein, in addition to acting as an autorepressor, acts as a transcriptional repressor of *proU*, which is involved in osmoregulation (UEGUCHI and MIZUNO 1993). It appears to repress transcription of other genes as well. From DNA-binding studies, it has been found that the H-NS protein binds to curved DNA and can function as a nonspecific repressor of gene expression (YAMADA *et al.* 1991; ZUBER *et al.* 1994). The negative effects of H-NS can be alleviated in at least two different ways. For example, CAP-cAMP seems to act as an anti-repressor of H-NS in the *pap* system that controls pilus synthesis (FORSMAN *et al.* 1992). A small RNA, DsrA, has been implicated as an antisilencer of H-NS in *rcsA* regulation that controls capsule polysaccharide synthesis (SLEDJESKI and GOTTESMAN 1995).

In the present work, we report the identification of a new gene, a mutation that leads to activation of *bgl* expression. The gene, *bglJ*, is located at min 99 of the *E. coli* genetic map. The mutant and wild-type *bglJ* genes have been cloned and sequenced. Genetic evidence and DNA sequencing analysis show that the mutant gene contains an IS*10R* insertion upstream of the *bglJ* gene, suggesting that it is the product of *bglJ* that leads to *bgl* activation. The preliminary characterization of *bglJ* is the subject of this paper.

MATERIALS AND METHODS

***bglJ4* mutant isolation and mapping of the mutation:** Spontaneous Bgl$^+$ mutants of laboratory strain JL191 were isolated at 30° by plating on MacConkey indicator medium containing salicin. Seventy-four separate Bgl$^+$ papillae were purified by streaking on the same medium. Cells were plated at 30° so that any conditional lethal mutations, which would be temperature-sensitive for growth, would be included. Six out of 74 showed a temperature-sensitive Bgl phenotype on salicin MacConkey medium but were not temperature-sensitive for growth on L medium. Table 1 contains a list of the strains used in this work.

Strain JL191 contains a Tn*10* in *tna*, a locus closely linked by P1 transduction to the *bgl* operon. Phage P1*vir* was grown on the mutant strains (MILLER 1972) and used to transduce a Bgl$^-$ strain RV to tetracycline resistance (Tc^r). After purification, the transductants were screened for their Bgl phenotype on salicin MacConkey medium. The presence of the transposon allowed rapid screening of those mutants containing mutations linked by P1 transduction to *tna* and presumably located in *bgl* or, less frequently, in *gyrB*. None of these six Ts mutants contained mutations that were linked to *tna* by P1 transduction. Although the mutants were isolated from independent papillae, the papillae were not necessarily from individual colonies, meaning that the mutants could be siblings. One mutant, JL1122, was chosen and the mutation, designated *bglJ4*, was further characterized.

To locate the *bglJ4* mutation on the *E. coli* genetic map, a Tn*10* linked to the mutation was isolated. First, the *tna*::Tn*10* of JL1122 was replaced with a *tna*$^+$ *bglB*::Tn*5* region from JL195 by P1 transduction. Kanamycin-resistant transductants were selected and then screened for their Bgl phenotype. Replacement of this region resulted in a Tc^s Bgl$^-$ phenotype. The presence of the *bglJ4* mutation was verified by growth on minimal medium containing arbutin. An Arb$^+$ phenotype requires an activated *bgl* operon, specifically the transport protein encoded by *bglF*. This strain construction resulted in a tetracycline-sensitive derivative of the *bglJ4* strain designated JL1122-1.

The isolation of a Tn*10* linked to *bglJ4* was obtained by preparing a pool of colonies with random Tn*10* insertions in the chromosome (KUMAMATO and BECKWITH 1983). Strain RV(*bglJ*$^+$) was infected with a λ phage containing Tn*10*, and Tc^r colonies were selected. These colonies were pooled and P1*vir* was grown on them. The P1 lysate was used to transduce strain JL1122-1 (*bglJ4*) to Tc^r. The transductants were screened for their Bgl phenotype. One Bgl$^-$ Tc^r transductant was found. Linkage of the Tn*10* to the *bglJ*$^+$ allele was ~90% as determined by P1 transduction. Subsequent transductions resulted in *bglJ4* Tn*10* derivatives in both the RV strain background and in the *pcn* strain background of MRi80. The *pcn* strain contains a mutation that lowers plasmid copy number, and this strain is often helpful in cloning and in complementation analysis (LOPILATO *et al.* 1986; LIU and PARKINSON 1989).

β-galactosidase assays: Strains MRi80 and MRi804 were ly-

TABLE 1

Bacterial strains, bacteriophages, and plasmids

Strain, phage, episome, or plasmid	Description	Source, reference, or construction
Bacterial strains		
RV	F^- Δ*lacX74 thi*	This laboratory
KO627	RV *bglR67*	This laboratory
JL191	RV *tna*::Tn*10*	This laboratory
JL195	RV*tna*$^+$ *bglB*::Tn*5*	This laboratory
JL1122	JL191 *bglJ4*	This work
JL1122-1	JL1122 *tna*$^+$ *bglB*::Tn*5*	P1*vir*.JL195 × JL1122 → Km^r Tc^s
JL236	RV*bglJ4 dnaC*(Ts) *zjj*::Tn*10*	This work
MC4100	F^- *araD139* Δ(*argF-lac*)U169 *rpsL150 relA1 flb5301 deoC1 ptsF25 rbsR*	This laboratory
MRi80	MC4100 *pcnB*	LOPILATO *et al.* (1986)
MRi804	MC4100 *pcnB bglJ4 zjj*::Tn*10*	This work
MRi844	MRi804 *serB*	This work
MRi844-1	MRi844 *recA*	This work
MRi844-2	MRi844 *recA nal*	This work
MRi844-3	MRi844 *nal*	This work
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac*[*F'proAB lacI^qZΔ M15*Tn*10*]^c	Stratagene Cloning Systems
Bacteriophages		
λM1	*bglR67-lacZ* fusion phage	LOPILATO and WRIGHT (1990)
λM12	*bglR*^{0}*-lacZ* fusion phage	LOPILATO and WRIGHT (1990)
Episomes		
F'104	PO1 of Hfr Hayes to *argF*	*E. coli* Genetic Stock Center
F'104-1	*bglJ*$^+$ *serB*$^+$	This work
Plasmids		
pBluescript II SK+	P_{lac}-*lacZ* cloning vector	Stratagene Cloning Systems
pBluescript II KS+	P_{lac}-*lacZ* cloning vector	Stratagene Cloning Systems
pJL1	*bglJ*$^+$, *Eco*RI-*Pst*I fragment in pSK	This work
pJL2	*bglJ4*, *Eco*RI-*Pst*I fragment in pSK	This work
pJL3	*bglJ4*, *Bam*HI- *Nhe*I fragment in pSK	This work
pJL4	*bglJ4*, *Bam*HI-*Nhe*I fragment in pKS	This work
pJL5	*Bcl*I fragment in pSK with P_{lac} proximal to *bglJ4*	This work
pJL6	*Bcl*I fragment in pSK with P_{lac} distal to *bglJ4*	This work
pJL7	*Hind*II-*Sma*I fragment of pJL3 in pSK	This work
pJL8	pJL3 Δ3-2	This work
pJL9	pJL3Δ6-1	This work

Km^r, resistance to kanamycin; Tc^s, sensitivity to tetracycline.

sogenized with a *bglR*^{0}*-lacZ* fusion phage, λM12 (LOPILATO and WRIGHT 1990). Care was taken to obtain single lysogens by repeatedly purifying MRi804 lysogens on L medium containing β-methyl glucoside (an inducer for *bgl*) and X-gal. Independent MRi80 lysogens were assayed to find the lysogens with the lowest activity that were presumably single lysogens. Four independent lysogens of MRi804 and two independent lysogens of MRi80 were obtained. The lysogenic cells were grown in M63 containing 0.4% succinate, 0.2% casamino acids, 0.1 mg/ml B1, and 1 mM $MgSO_4$. To induce *bgl* expression, 0.2% salicin was added. The *bglR67* strain KO627, containing a *bglR67-lacZ* fusion phage, λM1, was assayed as a control each time (LOPILATO and WRIGHT 1990). Assays were done on four separate occasions for the *bglJ*$^+$ strain and on three separate occasions for the *bglJ4* strain. The assay for β-galactosidase has been described previously (MILLER 1972).

Use of Kohara phages in cloning the *bglJ* gene: The Tn*10* linked to *bglJ4* was also linked to *dnaC* by P1 transduction. Using this Tn*10*, a mutant strain, JL236, was constructed that contained the *bglJ4 dnaC*(Ts) alleles. Strain JL236 was used in cloning the *bglJ* gene to detect double crossover events between the Kohara phages 672 and 673 (KOHARA *et al.* 1987; KOMINE *et al.* 1990), which carry the wild-type allele of *dnaC*, and the chromosome in the *bglJ-dnaC* region. Recombinants resulting from double crossover events were isolated by selecting for temperature-resistance, *dnaC*$^+$. Tetrazolium indicator medium containing arbutin was used to detect *bglJ*$^+$ recombinants that appeared as red sectors. Both Kohara phages 672 and 673 yielded *bglJ*$^+$ recombinants. From the overlapping region between phages 672 and 673, a 6.8-kb *Eco*RI-*Pst*I fragment was cloned onto the vector pBluescript II SK+ (Stratagene Cloning Systems). The *Eco*RI site is located in the vector DNA and the *Pst*I site in the chromosomal DNA of phage 673. When this plasmid, pJL1, was introduced into MRi804 *pcnB bglJ4*, wild-type recombinants were observed, meaning that the clone did in fact carry the *bglJ*$^+$ gene. By preparing DNA from these transformants, a plasmid, pJL2, carrying the *bglJ4* gene was obtained by homologous recombination. The frequency of recombination was not determined but was estimated at 10^{-3}–10^{-4}. This method of moving muta-

tions from the chromosome to a plasmid is applicable only when the mutant phenotype can be selected.

Complementation analysis: Complementation analysis was done under low plasmid copy number conditions by using the *pcn* strains MRi80 and MRi804. MRi80 contains the *bglJ*$^+$ gene, while MRi804 contains the *bglJ4* gene. The plasmids containing either the wild-type (pJL1) or mutant *bglJ* gene (pJL2) were introduced into the *pcn* strains by transformation and then were tested for their Bgl phenotype by plating on salicin minimal medium.

Complementation analysis was also carried out with a *rec* derivative of strain MRi844 and the episome F′104-1 described below. Using *zjj*::Tn*10,* which is linked to both the *bglJ4* and *serB* alleles, MRi844 was constructed by P1 transduction so that the episome (*serB*$^+$) could be selected. A *rec* derivative, MRi844-1, was constructed by transducing MRi844 with P1 grown on a *recA*::Tn*9* strain and selecting for chloramphenicol resistance. An initial conjugation experiment was done using the episome F′104, but the presence of the episome in MRi844-1 resulted in poor growth on glucose minimal and little if any growth on salicin minimal medium. To avoid any growth problems, a derivative of F′104 was used in complementation analysis. This derivative, F′104-1, was isolated from conjugants that produced larger colonies on glucose minimal medium, and it transfers only *bglJ*$^+$ and *serB*$^+$ and not *thr*$^+$ and *leu*$^+$ of original episome. A nalidixic acid-resistant (Nal^r) derivative, MRi844-2, was isolated by P1 transduction so that nalidixic acid could be used to select against the donor strain. From the mating of MRi844-1/F′104-1 with MRi844-2, SerB$^+$ Nal^r conjugants were selected on glucose minimal medium containing nalidixic acid, purified on the same medium, and then tested on salicin minimal medium to determine their Bgl phenotype. To verify that F′104-1 carried the *bglJ*$^+$ allele, a mating was done with MRi844-1/F′104-1 and a Nal^r derivative, MRi844-3 (Rec$^+$), again selecting for SerB$^+$ and Nal^r. After purifying these conjugants, the colonies were streaked on Salicin McConkey indicator medium and both Bgl$^-$ and Bgl$^+$ colonies were observed indicating the presence of the wild-type *bglJ* gene on the episome.

Subcloning and deletion analysis: Originally, the *bglJ4* gene was isolated on an *Eco*RI-*Pst*I fragment in pJL2. Figure 2 shows the relevant restriction enzyme sites of the DNA in the *bglJ4* region of the chromosome, and Table 1 contains the plasmids used in this work. A subclone of pJL2 was constructed by cloning the 1.7-kb *Bam*HI-*Nhe*I fragment into the *Bam*HI and *Spe*I sites of the pBluescript II SK+ and pBluescript II KS+ vectors to give pJL3 and pJL4. These vectors will subsequently be referred to as pSK and pKS. The *Bcl*I fragment was cloned into the *Bam*HI site of the pSK vector, which resulted in pJL5 and pJL6 differing only in the orientation of the *Bcl*I fragment. Plasmid pJL7 was constructed by cloning *Hin*cII-*Sma*I fragment from pJL3 into the *Eco*RV site of pSK. (The *Sma*I site is located in the vector DNA of pJL3.) All of these plasmids were introduced into MRi80 (*pcn bglJ*$^+$) by transformation, and their Bgl phenotypes were determined.

Deletions were constructed *in vitro* by using a combination of exonuclease and mung bean nuclease (New England Biolabs Exo-Size Deletion Kit). Plasmid DNA of pJL3 was digested with the restriction enzymes *Kpn*I to generate a 3′ extension and *Hin*dIII to give a 5′ extension. The linear DNA was treated with exonuclease that digests only from a 5′ extension. Samples were taken at 5-sec intervals for a total of 5 min. The reactions were terminated and the samples were treated with mung bean nuclease to generate blunt ends. Blunt end ligations were done by adding T4 DNA ligase to the samples and incubating them overnight at 16°. The ligation reaction mixes were introduced into strain XL-1 Blue (Stratagene Cloning Systems) by transformation. The transformants were purified, and the plasmid DNA was extracted and characterized by *Ssp*I restriction enzyme analysis resulting in pJL8 and pJL9. The endpoints of the deletions were determined by DNA sequence analysis as described below.

DNA sequence analysis of the *bglJ4* and *bglJ*$^+$ genes: The clones pJL3, which contains the 1.7-kb *Bam*HI-*Nhe*I fragment, and pJL7, which contains the *Hin*cII-*Sma*I fragment, were used in DNA sequence analysis. Primers that annealed to pSK DNA sequences as well as internal primers spanning the entire insert were used in dideoxynucleotide sequencing reactions with Sequenase (United States Biochemical, Amersham Life Science). In addition, primers homologous to each end of the insertion element IS*10R* were used to determine the adjacent chromosomal DNA sequences. Three sets of sequencing reactions were done for each primer using deoxyguanosine nucleotides, deazaguanosine nucleotides, and deoxyinosine nucleotides with terminal transferase (Stupi and Brummet 1991). The DNA sequence of both strands was determined.

The DNA sequence of the *bglJ*$^+$ gene was determined using a derivative of pJL1 that contained DNA from the *Eco*RI site to the *Bam*HI site with the primers and methods as described above. To determine the DNA sequence of the upstream region, a primer annealing to that region of the wild-type gene was utilized. Again, the DNA sequence of both strands was determined.

The DNA sequences obtained from the *bglJ4* and *bglJ*$^+$ clones were compared and analyzed to find an open reading frame using DNA Strider 1.0.1. Figure 3 shows the *bglJ* DNA sequence and the putative protein product. The DNA sequence of the *bglJ* gene has been assigned accession number U35834 in the GenBank database.

RESULTS

The *bglJ4* mutation results in a Bgl$^+$ phenotype: Spontaneous Bgl$^+$ colonies were isolated and purified as described in materials and methods. One of the Bgl$^+$ mutations was designated *bglJ4* and was further characterized. Linkage analysis using P1 transduction indicated that the *bglJ4* mutation was unlinked to loci in which mutations are known to result in *bgl* expression, namely *bgl, gyrA, gyrB,* and *hns.* Instead, the *bglJ4* mutation mapped to the min 99 region of the *E. coli* genetic map and was ~90% linked to the *dnaC* gene by P1 transduction.

To determine if the *bglJ* mutation affects *bgl* transcription, strains containing a *bglR^o-lacZ* fusion (*bglR^o* is nonactivated) and either the *bglJ4* or *bglJ*$^+$ alleles were assayed for β-galactosidase activity. The *bglJ4* mutant strain MRi804 had ~14 units of β-galactosidase activity uninduced and ~1600 units of activity when induced with salicin (Table 2). In comparison, the *bglJ*$^+$ strain MRi80 had 2 units of activity uninduced and 21 units when induced. Under inducing conditions, activity in the *bglJ4* mutant strain is 78% of that seen in *bglR67* strain KO627 that contains an activating point mutation in the CAP-cAMP binding site. Similar levels of expression were observed in the RV strain background (data not shown). Expression of the *bgl* operon in the *bglJ4* strain is occurring at the transcriptional level and the activity is sufficient that the mutant strain is phenotypically Bgl$^+$.

TABLE 2

Levels of *bgl* operon expression in *bglJ4* and *bglJ*$^+$ strains

Strain	β-galactosidase activity (Miller units)	
	− inducer	+ inducer
MRi80 *bglJ*$^+$ λM12	1.52 ± 0.56	21.4 ± 5.76
MRi804 *bglJ4* λM12	13.72 ± 1.87	1582 ± 307
KO627 λM1 control	6.76 ± 1.39	2035 ± 368

The control strain contains a *bglR67* mutation that results in the activation of the *bgl* operon.

TABLE 3

Complementation analysis: *bglJ4* is dominant to *bglJ*$^+$

Strain	Bgl phenotype
MRi844-2 *bglJ4 serB recA* + F′104-1 *bglJ*$^+$ *serB*$^+$	+
MRi804 *bglJ4* + pJL1 *bglJ*$^+$	+
MRi80 *bglJ*$^+$ + pJL2 *bglJ4*	+
MRi80 *bglJ*$^+$ + pJL1 *bglJ*$^+$	−

The plasmids used contained the *Eco*RI-*Pst*I fragment with and without the *bglJ4* mutation.

The *bglJ4* mutation is dominant: Identification of the *bglJ*$^+$ gene in the Kohara chromosomal library (KOHARA *et al.* 1987; KOMINE *et al.* 1990) was complicated by the fact that the wild-type gene confers no selectable phenotype, that is, a *bglJ*$^+$ strain is phenotypically Bgl$^-$. Therefore, a strain was constructed such that another gene carried on the same DNA fragment of the Kohara phage could be selected. Since the mutation was closely linked to *dnaC*, a strain JL236 was constructed containing the *dnaC*(Ts) and *bglJ4* alleles so that *dnaC*$^+$ recombinants could be selected. If the *bglJ* allele was carried on the same DNA fragment of the Kohara phage as the *dnaC*$^+$ allele, then *bglJ*$^+$ recombinants should occur as well. Both Kohara phages 672 and 673 yielded *bglJ*$^+$ recombinants. From the region of overlap between these two phages, a 6.8-kb *Eco*RI-*Pst*I DNA fragment was cloned into the vector pSK. This fragment was shown to contain the *bglJ*$^+$ allele by recombination as described in MATERIALS AND METHODS. Recombination *in vivo* was used in isolating a plasmid-borne *bglJ4* allele. The plasmid with the wild-type allele was first passaged through a strain containing the *bglJ4* allele and then plasmid DNA was isolated. Those rare plasmids that had undergone recombination between the chromosomal *bglJ4* and the plasmid *bglJ*$^+$ alleles were selected as Bgl$^+$ transformants on minimal medium containing salicin. Upon restriction enzyme analysis, the Bgl$^+$ transformants were found to contain a plasmid with the *bglJ4* mutation.

Complementation analysis was carried out in the *pcnB* strain that lowers the plasmid copy number. Strains were constructed so that the *bglJ4* mutation was located either on the chromosome or on a plasmid. In both constructions, the *bglJ4* mutation was dominant over *bglJ*$^+$ (Table 3). In addition, complementation analysis was done in a *bglJ4 rec*$^-$ derivative using a F′ episome with the *bglJ*$^+$ allele as described in MATERIALS AND METHODS. Mutant cells containing this episome retained their Bgl$^+$ phenotype indicating that the *bglJ4* mutation was dominant to *bglJ*$^+$ (Table 3). The presence of the mutation then results in a Bgl$^+$ phenotype.

Minimal size DNA fragment that results in a Bgl$^+$ phenotype: From the *Eco*RI-*Pst*I fragment with the *bglJ4* mutation, a 1.7-kb *Bam*HI-*Nhe*I fragment was subcloned in both orientations relative to the *lac* promoter (P_{lac}) of the vector by using pSK and pKS. Each clone, pJL3 and pJL4, was tested to determine its Bgl phenotype and both clones resulted in a Bgl$^+$ phenotype. The *Bam*HI-*Nhe*I fragment was further analyzed by additional subcloning and by constructing deletions (Figure 2). Deletions were generated *in vitro* and the resulting plasmid DNAs were introduced into MRi80 to determine their Bgl phenotypes. pJL8 contains deletion 3-2, which gave a Bgl$^+$ phenotype while pJL9 contains deletion 6-1, which gave a Bgl$^-$ phenotype. At a lower frequency, deletion 6-1 recombined to give Bgl$^+$ colonies, indicating that the mutation was still present. A fragment extending from the *Hinc*II site to the *Sma*I site of the pSK vector was cloned and tested in MRi80 for its Bgl phenotype. The *Hinc*II-*Sma*I clone, JL7, gave a Bgl$^-$ phenotype and did not yield Bgl$^+$ recombinants. This result, along with the data from the deletions, indicated that the *bglJ4* mutation must be located between the *Nhe*I and the *Hinc*II sites.

The *bglJ4* mutation is an IS*10R* insertion mutation required for the Bgl$^+$ phenotype: Restriction enzyme analysis showed that the *Eco*RI-*Pst*I DNA fragment of pJL2 that contains the *bglJ4* allele was ~1 kb larger than the fragment of pJL1 that contains the *bglJ*$^+$ allele (data not shown). This increase in fragment size suggested the presence of an insertion element in the *bglJ4* clone.

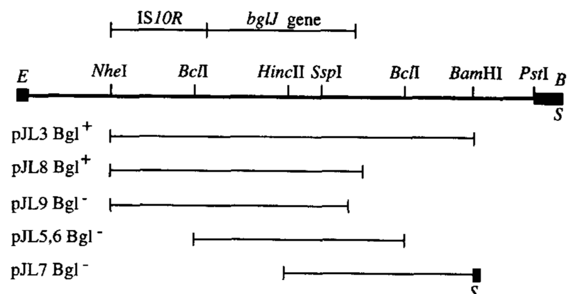

FIGURE 2.—Relevant restriction enzyme sites in the *bglJ4* region. The relevant restriction enzyme sites are shown for the DNA of the *bglJ4* region of the chromosome. The plasmids above are subclones of pJL3 and contain the DNA fragments shown. pJL8 and pJL9 are deletion derivatives of pJL3, and the remaining DNA carried by the plasmids is given. The Bgl phenotypes of these plasmids are included. The restriction enzyme sites of the vector DNA are represented by *B*, *Bam*HI; *S*, *Sma*I; *E*, *Eco*RI.

```
  1  AACTTAACTTGCCTTCTGTTTATGGAGATGCCGCAGAATGGAACACAGCCGAATTAAGAA   60
                                             M  E  H  S  R  I  K  K
 61  GAGAAATGTCGCACTCATAGAAAAATGCGTCATGAGTAGTATCGGTATTGAGAGTTTATT  120
      R  N  V  A  L  I  E  K  C  V  M  S  S  I  G  I  E  S  L  F
121  CAGAAAGTTTGCGGGTAACCCTTATAAGCTCCATACCTATACCAGTCAGGAGTCATTTCA  180
      R  K  F  A  G  N  P  Y  K  L  H  T  Y  T  S  Q  E  S  F  Q
181  GGATGCCATGTCGCGGATCTCGTTTGCGGCGGTCATTTTTCTTTTTCTGCCATGAGAAG  240
      D  A  M  S  R  I  S  F  A  A  V  I  F  S  F  S  A  M  R  S
241  TGAGCGCAGAGAGGGATTATCTTGCCTGACTGAACTGGCGATTAAGTTTCCGCGTACCCG  300
      E  R  R  E  G  L  S  C  L  T  E  L  A  I  K  F  P  R  T  R
301  GCGTTTAGTTATTGCGGATGATGATATTGAAGCTCGGCTGATTGGTTCATTGTCGCCATC  360
      R  L  V  I  A  D  D  D  I  E  A  R  L  I  G  S  L  S  P  S
361  ACCGCTGGACGGTGTATTAAGTAAAGCGTCAACGCTGGAGATTTTTCATCAGGAACTCTT  420
      P  L  D  G  V  L  S  K  A  S  T  L  E  I  F  H  Q  E  L  F
421  TTTGTCATTAAATGGTGTACGTCAGGCGACCGACCGACTGAACAATCAGTGGTACATTAA  480
      L  S  L  N  G  V  R  Q  A  T  D  R  L  N  N  Q  W  Y  I  N
481  CCAAAGCCGGACGTTAAGCCCGACGGAGAGAGAAATATTGCGCTTTATGTCGCGTGGCTA  540
      Q ,S [R  T  L  S  P  T  E  R  E  I  L  R  F  M  S  R  G  Y]
541  CTCAATGACACAAATTGCCGAGCAGCTTAAACGCAATATCAAAACGATCCGTGCACATAA  600
      S  M  T  Q  I  A  E  Q  L  K  R  N  I  K  T  I  R  A  H  K
601  ATTTAATGTGATGTCGAAACTGGGCGTCAGTTCTGACGCAGGGTTGTTGGAGGCCGCAGA  660
      F  N  V  M  S  K  L  G  V  S  S  D  A  G  L  L  E] A  A  D
           ▼
661  TATTCTGTTATGTATGCGGCATTGCGAAACAAGTAATGTGTTGCATCCCTATTAATCCGC  720
      I  L  L  C  M  R  H  C  E  T  S  N  V  L  H  P  Y  OCH
721  ATGATGCCGGGTTTACTTCCCCGGCAGTGCTTTCATTTCAGCGTACAATCGCCACATTGC  780

781  TGCACATCCGGTAAGCGATAACGCTGGCAGCAAGTGCGGCGCACCAGCAGGCCGTCGCGC  840
        ▼
841  AGT
```

FIGURE 3.—Nucleotide sequence of the *bglJ* gene. Nucleotides 1–9, boxed above, are duplicated in DNA isolated from a *bglJ4* strain that contains an IS*10R* insertion upstream of nucleotide 1. A potential translational start codon at nucleotide 38 is shown, and the amino acids are given following the first methionine. Amino acids 151–205 are boxed to show the region of homology to the UhpA-LuxR family of transcriptional activators as in STOUT *et al.* (1991). ▲, the endpoints of deletion 6-1 at nucleotide 667 and deletion 3-2 at nucleotide 843.

DNA sequence analysis, described below, identified the insertion as IS*10R* orientated such that the IS*10R* promoter, P_{out}, is upstream of the only open reading frame that is presumably the *bglJ* gene. The orientation of the IS*10R* raised the possibility that transcription could be initiating from the insertion element since expression from P_{out} can result in gene activation (CIAMPI *et al.* 1982; SIMONS *et al.* 1983: WANG and ROTH 1988). To determine whether IS*10R*, and specifically P_{out}, is required for the *bglJ4* phenotype, a *BclI* fragment was cloned from pJL3 (Figure 2). This fragment contains the entire *bglJ* gene and 65 bp of IS*10R* but lacks P_{out}. The *BclI* fragment was cloned in two orientations into the *Bam*HI site of the plasmid vector pSK such that the P_{lac} promoter of pSK was either adjacent or distal to the IS*10R* sequences. These clones, pJL5 and pJL6, were introduced into MRi80 and both clones resulted in a Bgl$^-$ phenotype as determined on salicin minimal medium. The P_{out} promoter is necessary, then, for the *bglJ4* phenotype suggesting that *bglJ* expression is required for the phenotype and that this *BclI* fragment does not contain another promoter. Interestingly, the clone with the vector P_{lac} adjacent to the remaining IS*10R* sequences, *i.e.*, in the same orientation as IS*10R* is found in *bglJ4*, was phenotypically Bgl$^+$ on MacConkey indicator medium containing salicin even though it was Bgl$^-$ on salicin minimal medium. Growth on salicin minimal medium requires a higher level of expression of the *bgl* operon than that required for a positive phenotype on MacConkey indicator medium. The clone with P_{lac} located distal to the IS*10R* sequences gave a Bgl$^-$ phenotype on the same indicator medium. The phenotypes conferred by these plasmids provide *in vivo* evidence for the direction of transcription of the *bglJ* gene and suggest that *bglJ* is expressed both from P_{out} and P_{lac} but that expression from the former promoter in this context is stronger. Thus, the level of expression of the *bglJ* product determines the level of expression of the *bgl* operon. The need for *bglJ* expression is consistent with the dominance of the *bglJ4* mutation.

DNA sequence analysis: Using the strategy of primer walking, DNA sequence analysis of the 1.7-kb *Bam*HI-*Nhe*I fragment was undertaken. Sequencing revealed the presence of an insertion, described above, with the *Nhe*I site from IS*10R* (HALLING *et al.* 1982). To determine if the original clone, pJL2, contained the entire insertion element, a primer homologous to the opposite end of IS*10R* (inside end) was used to sequence a derivative of pJL2 that contained DNA from the *Eco*RI to the *Bam*HI site. Sequencing from this end showed the presence of 73 bp from IS*10R* (HALLING *et al.* 1982). Taken together, the IS*10R* sequences of both ends and the increase in size of the mutant clone imply that the entire IS*10R* is present. In addition, when the wild-type *bglJ* gene was sequenced, comparison of the mutant and wild-type DNA sequences revealed a 9-bp duplication adjacent to the IS*10R* that suggests that the entire insertion element has transposed to a site upstream of the *bglJ* gene.

To determine how far from the insertion sequences the *bglJ* gene extends, the endpoints of two deletions were determined by DNA sequence analysis. Deletion 3-2 is Bgl$^+$ and its endpoint was located at position 843 bp that meant that the entire gene is contained on that fragment. Deletion 6-1 has a Bgl$^-$ phenotype and its endpoint is located at position 667 bp. The *bglJ* gene must end then between nucleotides 667 and 843, which are the endpoints of the two deletions (Figure 3).

When the DNA sequence from position 1 to position 843 was translated in all six possible reading frames, five contained numerous stop codons. Only one reading frame had a region that could be translated into a protein (Figure 3). If transcription initiates from the IS*10R*, then translation could begin at the first methionine codon producing a protein of 225 amino acids. The orientation of the open reading frame is consistent with the orientation of the gene predicted from the genetic evidence above. This suggests that the open reading frame encodes the BglJ protein. The putative protein contains 1.6 times more basic amino acids than acidic amino acids. There are homologies in the range of 28–48% identity and from 48 to 70% similarity between amino acids 151–205 in this protein and several other regulatory proteins in *E. coli, Bacillus, Salmonella,* and *Erwinia* (ALTSCHUL *et al.* 1990). Amino acids 151–

205 show 48% identity and 70% similarity to amino acids found in the carboxy terminus region of the RcsB protein, a positive regulator of capsule polysaccharide synthesis (ALTSCHUL *et al.* 1990; STOUT *et al.* 1991). RcsB protein is a member of the UhpA-LuxR family of regulatory proteins (FRIEDRICH and KADNER 1987; HENIKOFF *et al.* 1990; STOUT *et al.* 1991). The carboxy terminus contains a potential helix-turn-helix motif that is shared by the BglJ and RcsB protein. The search for proteins with homology to the putative BglJ protein yielded other members of this family including FimZ, UvrC, MalT, RcsA, NarP, and NarL (ALTSCHUL *et al.* 1990; STOUT *et al.* 1991; SWENSON and CLEGG 1992).

DISCUSSION

The *bglJ4* mutation, which has been characterized as an IS*10R* insertion upstream of the *bglJ* gene, results in *bgl* expression. The amount of *bgl* expression is 78% of a fully induced strain containing an activating point mutation in the CAP-cAMP-binding region. The *bglJ* gene has been identified as *o225a* (BURLAND *et al.* 1995) and *yjjR* at 4602.8 kb (EcoMap8, K. RUDD, personal communication).

The *bgl* operon is silent, and the molecular mechanism for this silencing is unknown. A region upstream of the CAP-cAMP-binding site, first identified as the target site for activating insertion sequences, is involved in the silencing since its deletion results in activation of the *bgl* operon. Point mutations within the CAP-cAMP-binding site that increase affinity for CAP-cAMP also lead to activation suggesting that the silencing region might function by somehow interfering with the normal CAP-cAMP-DNA interaction. Insertions of IS*1*, IS*5*, or synthetic oligonucleotides also lead to activation either by disrupting the silencing region or by increasing its distance from the promoter. Deletions and insertions thus appear to act in an analogous way by disrupting the silencing region. Activation by insertion sequences, either IS*1* or IS*5* leads to significantly higher levels of bgl operon expression than does simple deletion of the silencing region. Perhaps the higher levels seen with IS activation are due both to disruption of the silencer and to enhancement of *bgl* transcription by IS-specific proteins. Ins5A, which is encoded by IS*5*, has been reported to play a role in *bgl* activation when IS*5* is located either upstream or downstream of the *bgl* promoter (SCHNETZ and RAK 1992).

Mutations in DNA gyrase and in the DNA-binding protein H-NS are known to activate the *bgl* operon. Though the precise mechanism is not known, it is likely that both types of mutations lead to local alterations of DNA supercoiling and/or structure. The lower levels of operon expression seen with those mutations (at least with the particular alleles used) compared to insertion or deletions activating mutations suggest that they may not activate by the same mechanisms. Our characterization of the *bglJ4* mutation identifies yet another activator of the *bgl* operon.

Further characterization of the *bglJ* gene and the protein it encodes will reveal more concerning its role in *bgl* expression. The putative protein encoded by the *bglJ* gene shows homology to the UhpA-LuxR family of regulatory proteins and most closely shares homology with the positive regulator of polysaccharide synthesis, RcsB (ALTSCHUL *et al.* 1990; HENIKOFF *et al.* 1990; STOUT *et al.* 1991). Consistent with its role as a regulatory protein is the basic nature of the protein predicted from the *bglJ* DNA sequence. It may act, then, as a type of regulatory protein on *bgl* operon expression. The BglJ protein could directly modulate *bgl* expression or could act indirectly through other components that are known to affect *bgl* expression such as H-NS or DNA gyrase.

Properties of the *bglJ4* mutation suggest that the BglJ protein is necessary for *bgl* activation. The dominance of the mutation to wild type suggests a gain of function in the mutant strain that results in a Bgl$^+$ phenotype. This dominance is also consistent with the nature of the mutation. The *bglJ4* mutant strain contains an IS*10R* upstream of the gene and the presence of the insertion is necessary for *bgl* expression. The upstream location of the IS*10R* suggests that the insertion most likely increases the amount of *bglJ* product. The IS*10R* could increase the expression of the *bglJ* gene by providing a promoter, *i.e.*, P_{out}, or by enhancing transcription from a promoter already present. Further analysis of the expression of the *bglJ* gene will determine at what level it is expressed.

While the *bglJ4* mutant was isolated as exhibiting a temperature-sensitive Bgl phenotype, the temperature-sensitivity may not be related necessarily to *bglJ*, but instead to the *bgl* operon. Temperature-sensitivity of phospho-β-glucosidase B, the product of the *bglB* gene, has been reported in certain strains (SCHAEFLER and MAAS 1967; WILSON and FOX 1983). It is possible that the presence of the *bglJ4* mutation somehow affects the stability of the BglB protein resulting in a temperature-sensitive Bgl phentoype. Alternatively, the BglJ protein itself may be temperature-sensitive or it could affect a process, *i.e.*, gene silencing, that is temperature dependent. It is unlikely that *bglJ* expression is temperature-sensitive since transcription may be initiating either from the IS*10R* or is enhanced by the insertion.

Finally, experiments to inactive *bglJ* will reveal whether it is a gene essential for cell viability. The *bglJ* gene may well have more general effects in the cell and not be specific for *bgl* activation. Indeed, *bgl* activation occurs in *bglJ*$^+$ strains, implying that the *bglJ4* allele is not required for *bgl* expression caused by mutations upstream of the *bgl* operon or by mutations located in other genes such as *gyr* and *hns*. Mutations in the *gyr* genes or in the *hns* gene affect not only *bgl* expression but have pleiotropic effects on the cell. Further analysis

of *bglJ* gene and the role of its product may reveal additional functions and provide new perspectives on the silencing and the activation of the *bgl* operon.

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