CRYPTIC OPERON FOR β-GLUCOSIDE METABOLISM IN *ESCHERICHIA COLI* K12: GENETIC EVIDENCE FOR A REGULATORY PROTEIN[1]

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Manuscript received August 5, 1980
Revised copy received December 8, 1980

ABSTRACT

Escherichia coli K12 does not metabolize β-glucosides such as arbutin and salicin because of lack of expression of the *bglBSRC* operon, which contains structural genes for transport (*bglC*) and hydrolysis (*bglB*) of phospho-β-glucosides. Mutants carrying lesions in the *cis*-acting regulatory site *bglR* metabolize β-glucosides as a consequence of expression of this cryptic operon (PRASAD and SCHAEFLER 1974). We isolated mutations promoting β-glucoside metabolism that were unlinked to *bglR*; some of these mutations were shown to be amber. All of them were mapped at 27 min on the *E. coli* K12 linkage map and appeared to define a single gene, for which we propose the designation *bglY*. Utilization of β-glucosides in *bglY* mutants appeared to be a consequence of expression of the *bglBSRC* operon, since *bglB bglR* and *bglB bglY* double mutants had the same phenotype. All *bglY* mutations analyzed were recessive to the wild-type *bglY*+ allele. Phospho-β-glucosidase B and β-glucoside transport activities are inducible in *bglY* mutants, as they are in *bglR* mutants. Metabolism of β-glucosides in both *bglR* and *bglY* mutants required cyclic AMP. We propose that *bglY* encodes a protein acting as a repressor of the *bglBSRC* operon, active in both the presence and absence of β-glucosides, whose recognition site would be within the *bglR* locus.

THE ability to utilize β-glucosides is not uniform among microorganisms (SCHAEFLER and MALAMY 1969; SCHAEFLER, MALAMY and GREEN 1969). The phenotypic spectrum ranges from strains that are able to metabolize aromatic as well as aliphatic β-glucosides (*e.g.*, Enterobacter strains) to strains that are unable to metabolize any known β-glucoside (*e.g.*, *Escherichia coli*, Salmonella and Shigella strains). These compounds are taken up in a 6-phosphorylated form and cleaved by phospho-β-glucosidase activities (FOX and WILSON 1968; SCHAEFLER and MALAMY 1969; SCHAEFLER, MALAMY and GREEN 1969).

Special attention has been paid to the Enterobacteriaceae, which are unable to metabolize any known β-glucoside. From these strains, it is possible to isolate mutants that can metabolize β-glucosides and that, therefore, exhibit functions of a normally dormant metabolizing system. The structure of such cryptic systems and the type of signals that turn them on have been studied in *E. coli* K12. Spon-

[1] A preliminary account of this work has been reported (DEFEZ, R. and M. DE FELICE, 25th Meeting of the Associazione Genetica Italiana, Padova, Italy, October 1979).

taneous β-glucoside-utilizing mutants (*bgl*$^-$) of this organism can be selected in minimal medium containing arbutin or salicin as the sole carbon source. The genes for transport (*bglC*) and hydrolysis (*bglB*) of 6-phosphorylated β-glucosides, which are cryptic in the wild type, become expressed in these mutants; the presumed structural gene (*bglS*) for a diffusible factor responsible for the induction of the system also appears to be expressed. PRASAD and SCHAEFLER (1974) have shown that, in the cases they analyzed, the expression of this system is a consequence of mutations in a site located between *bglS* and *bglC*, named *bglR*, which is suggested by these authors to represent a *cis*-acting regulatory region from which a bidirectional transcription of the structural genes originates. This region, inactive in the wild type, would be activated by mutation. The *bglBSRC* operon is located at 83 min on the *E. coli* K12 linkage map (BACHMANN and LOW 1980). Another gene, *bglA*, lying outside the *bglBSRC* operon, is expressed constitutively and codes for phospho-β-glucosidase A, an enzyme that is able to hydrolyze arbutin but not salicin (PRASAD, YOUNG and SCHAEFLER 1973); whereas, the cryptic, *bglR*-controlled, phospho-β-glucosidase B activity is able to hydrolyze both arbutin and salicin. In spite of the presence of constitutive β-glucosidase A activity, wild-type *E. coli* K12 does not utilize arbutin because this compound is not transported into the cell, since the only known gene for β-glucoside uptake is the cryptic *bglC* gene. Double *bglR bglC* mutants are unable to metabolize β-glucosides, because uptake is impaired. Double *bglR bglB* mutants utilize arbutin, but not salicin, because phospho-β-glucosidase B is impaired and phospho-β-glucosidase A is active.

While the model outlined above predicts that all β-glucoside-utilizing mutants should be altered in the *bglR* locus, occasionally such mutants are found not to be co-transducible with markers located at 81 to 83 min (unpublished results and personal communication from D. L. HARTL). Therefore, we undertook a genetic analysis aimed at identifying possible unknown elements of the *bgl* system. In the present work, we show that utilization of β-glucosides can be the consequence of mutations in a previously unknown gene, for which we propose the designation *bglY*. This gene carries genetic information for a protein whose activity is responsible for the silent condition of the *bglBSRC* operon.

In this paper, the phenotypic symbols Bgl$^+$ and Bgl$^-$ refer to strains that are able and not able to metabolize β-glucosides, respectively; these symbols must not be confused with the *bgl* designation of genotype. For example, a strain that utilizes β-glucosides will be indicated as Bgl$^+$ when we refer to the phenotype, and as *bgl*$^-$, when we refer to the genotype.

MATERIALS AND METHODS

Bacterial strains: The *E. coli* K12 strains used are described in Table 1.

Strain DF29d (F123 *cysB*+ *bglY207* / *cysB bglY207*) was prepared according to the following procedure. Strain DF29b (F123 *cysB*+*bglY*+ / *cysB bglY207 his-68*) was infected with P1 phage grown on strain FS29a (*cysB bglY207*). Bgl$^+$ transductants were selected and scored for the external marker, *cysB*. Only strains that had inherited from the donor both the *bglY207* allele and the *cysB* mutation were taken in consideration, because they could not be considered revertants. We purified one of them and named it DF29c (F123 *cysB bglY207* / *cysB bglY207 his-68*). In

order to transfer the episome, this strain was cross-streaked against strain FS29c (*cysB trp-45 bglY207*) with selection of Trp+ (in the absence of histidine to select against the donor). All the diploids obtained showed a Bgl+ Cys- phenotype, which demonstrated that strain DF29c contains the *bglY207* mutation on both the chromosomes and the episome. We prepared a spontaneous derivative of strain DF29c having a *cysB*+ allele on the F factor (this was tested by mating it with an F- *cysB*). This strain was named DF29d.

Strain FA31b (*bglY202 bglB208 cya-283*) was isolated through a cross between P1 phage grown on strain MI292b (*rbs*+ *cya-283*) and strain FA31 (*rbs-115 bglY202 bglB208*). Rbs+ transductants were selected and scored for utilization of arbutin in the presence of cyclic AMP (see Figure 1).

Media and growth conditions: Minimal medium was as described by VOGEL and BONNER (1956). Carbon sources were added at a final concentration of 0.4% (glucose) or 0.2% (other carbon sources). Supplements, when required, were added at a final concentration of 50 μg per ml), with the exception of thiamine, cystein and histidine (10 μg per ml). Minimal plates contained the same minimal medium with the addition of 20 g Bacto-agar per liter.

The LC broth contained per liter: 8 g NaCl, 10 g Bacto-tryptone, 5 g yeast extract, 0.3 g $CaCl_2$, 4 g glucose. Tryptone-rich medium plates contained, per liter: 5 g NaCl, 10 g Bacto-tryptone, 20 g Bacto-agar. EHA soft-agar medium contained, per liter: 8 g NaCl, 3 g glucose, 2 g sodium citrate, 13 g Bacto-tryptone, 7 g Bacto-agar. CEM plates were a modification of the EMB plates (MILLER 1972) having the following composition: 3 g Bacto-casamino acids, 0.4 g Bacto-eosin, 65 mg Bacto-methylene blue, 20 g Bacto-agar, 5 g NaCl, 2 g K_2HPO_4, 2 g arbutin, per liter H_2O.

All microbiological reagents were from Difco laboratories. Other chemicals were of the highest purity commercially available, mostly from Sigma Chemical Co., St. Louis, MO., U.S.A.

The rate of utilization of salicin and arbutin was comparable when cells were grown at 30°; at 37°, utilization of arbutin in many Bgl+ mutants was much faster. This was probably a consequence of the previously reported temperature sensitivity of β-glucosidase B (SCHAEFLER and MAAS 1967). To avoid confusion due to this property, we grew cells at 30° when analyzing the Bgl phenotype, and at 37° in all the other cases.

Isolation of mutants: Spontaneous mutants were selected at 30° by spreading on either arbutin or salicin plates 10^8 exponentially growing cells. In the procedure for isolating independent mutants from strain PS1079, each plate was seeded with cultures deriving from different clones and only one colony was picked from each plate. The mutants isolated from strain Ca85 were not necessarily independent of each other, since several colonies were picked from each plate. Fifty to 80 mutants per plate appeared after 48 hr of incubation.

Nitrosoguanidine-induced mutants were isolated according to MILLER (1972). Bgl+ clones were selected both on arbutin and on salicin plates and appeared at a frequency 5-fold higher than from nonmutagenized cultures. Also, in this case, several colonies were picked from each original culture.

Test for amber mutations: 10^8 cells of each mutant were suspended in 2 ml of EHA soft agar and plated on tryptone-rich medium plates. After drying the soft agar, a drop of *φ80psu3* (ANDOH and OZEKI 1968) was added in the center of each plate, which was then incubated. After growth, single colonies from the lysis area were purified and lysogens were selected on the basis of their ability to produce the same phage and to allow growth of T4 phage containing the mutation *amB17* in gene *23* (obtained from A. COPPO and J. F. PULITZER). The Bgl phenotype of the lysogens was then tested on arbutin and salicin plates.

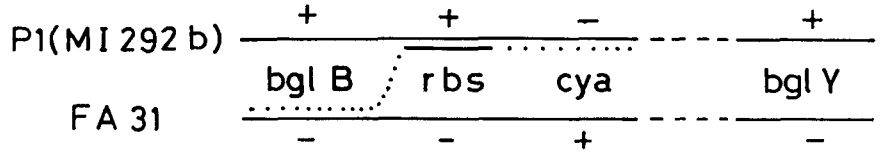

FIGURE 1.—Transductional cross for isolation of strain FA31b.

TABLE 1

*Bacterial strains**

Strain	Genotype	Origin or reference
Ca85	HfrH *thi-1 lac* amber	J. BECKWITH
H677	F⁻ *thi-1 his-68 tyrA2 trp-45 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	Coli Genetic Stock Center
HSSP-212	F⁻ *cysB pyrF tna-2*	R. SOMERVILLE
KLF23/KL181	F123 (*cysB*+ *trp*+ *bglY*+ *galU*+)/*pyrD34 trp-45 his-68 recA1 thi-1 galK35 xyl-7 mtl-2 malA1 rpsL118*	Coli Genetic Stock Center
PS1079	F⁻ *ara rbs-115 xyl-7 lacY1 mglP1*	M. LEVINTHAL
PS1479	F⁻ *xyl-7 lacY1 mglP1 ilvD530 leu*	M. LEVINTHAL
W1692-YS57/ Fcol	F(col V,B *cys*+ *trp*+ *tonB*+)/ *his pro* Δ(*tonB-trp*)	C. YANOFSKY
W3110	wild type	C. YANOFSKY
W3110 Δ(*tonB-trpAE1*)	Δ(*tonB-trpAE1*)	C. YANOFSKY
MI292b	F⁻ *cya-283 gal*	M. IACCARINO
MI324b	F⁻ *thi-1 his-68 tyrA2 cysB pyrF purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *cysB*⁻ *pyrF*⁻ derivative of H677 with P1 (HSSP-212)
MI324c	F⁻ *thi-1 his-68 tyrA2 cysB trp-45 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*pyrF*+ *trp*⁻ derivative of MI324b with P1 (H677)
MI324d	F⁻ *thi-1 his-68 tyrA2 pyrF trp-45 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*cysB*+ *trp*⁻ derivative of MI324b with P1 (H677)
MI385b	F⁻ *ilvD bglR20 cya-283*	M. IACCARINO
FA31	F⁻ *ara rbs-115 xyl-7 lacY1 mglP1 bglB208 bglY202*	See RESULTS
FA31a	F⁻ *thi-1 his-68 tyrA2 cysB bglY202 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *bglY202* derivative of MI324c with P1 (FA31)
FA31b	F⁻ *ara cya-283 xyl-7 lacY1 mglP1 bglB208 bglY202*	see MATERIALS AND METHODS
FA31c	F⁻ *thi-1 tyrA2 cysB bglY202 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	His+ derivative of FA31a
FA35	HfrH *thi-1 lac* amber *bglY203*	See RESULTS
FA35a	F⁻ *thi-1 his-68 tyrA2 cysB bglY203 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *bglY203* derivative of MI324c with P1 (FA35)
FA35c	F- *this-1 tyrA2 cysB bglY203 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	His+ derivative of FA35a
FA37	HfrH *thi-1 lac* amber *bglY204*	See RESULTS
FA37a	F⁻ *thi-1 his-68 tyrA2 cysB bglY204 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *bglY204* derivative of MI324c with P1 (FA37)
FA37c	F⁻ *thi-1 tyrA2 cysB bglY204 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	His+ derivative of FA37

TABLE 1—Continued

Strain	Genotype	Origin or reference
FA39	HfrH *thi-1 lac* amber *bglY205*	See RESULTS
FA39a	F⁻ *thi-1 his-68 tyrA2 cysB bglY205 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *bglY205* derivative of MI324c with P1 (FA39)
FA39c	F⁻ *thi-1 tyrA2 cysB bglY205 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	His+ derivative of FA39a
FS20	F⁻ *ara rbs-115 xyl-7 lacY1 mglP1 bglY206*	See RESULTS
FS20a	F⁻ *thi-1 his-68 tyrA2 cysB bglY206 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *bglY206* derivative of MI324c with P1 (FS20)
FS20b	F⁻ *ara cya-283 xyl-7 lacY1 mglP1 bglY206*	*rbs*+ *bglB*+ *cya-283* derivative of FS20 with P1 (FA31b)
FS20c	F⁻ *thi-1 tyrA2 cysB bglY206 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	His+ derivative of FS20a
FS29	F⁻ *ara rbs-115 xyl-7 lacY1 mglP1 bglY207*	See RESULTS
FS29a	F⁻ *thi-1 his-68 tyrA2 cysB bglY207 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*trp*+ *bglY207* derivative of MI324c with P1 (FS29)
FS29b	F⁻ *thi-1 his-68 tyrA2 cysB trp-45 bglY207 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	*pyrF*+ *cysB*⁻ *bglY207* derivative of MI324d with P1 (FS29a)
FS29c	F⁻ *thi-1 tyrA2 cysB trp-45 bglY207 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	His+ derivative of FS29b
DF29b	F123 (*cysB*+ *trp*+ *bglY*+ *galU*+)/ *thi-1 his-68 tyrA2 cysB trp-45 bglY207 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	Derivative of FS29b containing the F123 episome
DF29c	F123 (*cysB*⁻ *trp*+ *bglY207 galU*+)/*thi-1 his-68 tyrA2 cysB trp-45 bglY207 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	see MATERIALS AND METHODS
DF29d	F123 (*cysB*+ *trp*+ *bglY207 galU*+)/*thi-1 his-68 tyrA2 cysB trp-45 bglY207 purC50 lacY1 gal-6 xyl-7 mtl-2 malA1 strA125 tonA2 tsx-70*	see MATERIALS AND METHODS

* Genetic symbols are those used by BACHMANN and Low (1980).

ϕ80psu3 lysogens of strains Ca85 (containing a *lac* amber mutation) were obtained by selecting Lac+ colonies from crosses between the phage and the bacterial strain. The check of the lysogenic nature of the derivatives and the analysis of the Bgl phenotype were performed as for the PS1079 derivatives.

Only those mutants whose ability to utilize β-glucosides was lost after lysogenization were considered to carry amber mutations. A further check of these amber mutations consisted of curing with UV the lysogens from *ϕ80psu3* and showing that the restoration of sensitivity to the same phage was accompanied by reacquisition of the Bgl+ phenotype.

Transductions: Transductions were performed with P1CM*clr*100 (ROSNER 1972), as described by GUARDIOLA *et al.* (1974).

Episome transfer: Matings on CEM plates are described in the RESULTS. Matings in liquid media were performed by mixing donor and recipient in a ratio of 1 to 10 from cultures in LC broth and incubating for 90 min before washing and plating dilutions of the original cell mixtures on selective plates.

Preparation of colicins: Colicins V and B were prepared from strain WI692-YS57/Fcol according to SPUDICH, HORN and YANOFSKY (1970).

Enzyme assay: Phospho-β-glucosidase B activity was assayed in whole cells with salicin as a substrate according to PRASAD and SCHAEFLER (1974). Specific activities are expressed as nmoles of product per min per mg (dry weight) of cells.

Transport assay: The qualitative estimation of β-glucoside uptake was based on the method reported by PRASAD and SCHAEFLER (1974). About 200 colonies were grown on succinate minimal plates for 48 hr without (noninducing conditions) and with (inducing conditions) 5×10^{-3} M β-methyl-D-glucoside. The uptake of p-nitrophenyl-β-D-glucoside was tested by laying 1 ml of a 4×10^{-2} M solution of this compound on the surface of each plate. Colonies that expressed β-glucoside transport turned yellow within 5 to 10 min, due to rapid incorporation of p-nitrophenyl-β-D-glucoside, followed by cleavage of this compound and production of p-nitrophenol. Colonies that did not express β-glucoside transport did not turn yellow within 4 hr.

The quantitative method used in the experiment of Figure 2 was as follows. To cells growing exponentially in minimal medium with and without inducer (5×10^{-3} M β-methyl-D-glucoside) was added p-nitrophenyl-β-D-glucoside (2×10^{-3} M final concentration) and incubated with aeration for 1 hr. During this time, samples were withdrawn, chilled for a few minutes and centrifuged at 4°; the content of p-nitrophenol liberated into the medium was detected spectrophotometrically at 410 nm.

RESULTS

Isolation of mutants: We isolated *E. coli* K12 Bgl^+ mutants both spontaneous (from strains PS1079 and Ca85) and induced by nitrosoguanidine mutagenesis (from strain PS1079). From strain PS1079, we isolated 28 independent spontaneous mutants and 300 nitrosoguanidine-induced mutants; from strain Ca85, we isolated 240 spontaneous mutants. Half of these mutants were obtained from arbutin plates and half from salicin plates. All of the mutants were able to utilize both compounds, with the exception of one of the nitrosoguanidine-induced ones (FA31), which utilizes arbutin but not salicin.

Among the mutations analyzed in this work, only three of those isolated in strain Ca85 were suppressed after lysogenization with *φ80psu3* and reappeared after curing the phage from the cells, thus demonstrating their amber nature. We have tentatively assigned distinct allele numbers (see Table 3) to these mutations, although it is not certain that they are all independent.

Identification of a new regulatory gene bglY: In order to test for the presence of mutations in the *bglBSRC* operon, P1 phage was grown on each of the 28 spontaneous Bgl^+ derivatives of strain PS1079, the nitrosoguanidine-induced mutant FA31 and the three strains containing amber mutation, and crossed with strain PS1479 (*ilvD530*): Ilv^+ transductants were selected and scored for the ability to utilize β-glucosides. For each transduction, we screened a minimum of 40 and a maximum of 200 transductants. The results can be summarized as follows: (1) 26 of the 28 independent spontaneous mutants isolated from strain PS1079 showed

TABLE 2

Identification of a bglB *mutation in strain FA31**

Classes found	Genotype			No. of Bgl+ transductants
Utilizers of arbutin only	*bglR*+ *bglB*−			47/300 (15.7%)
Utilizers of both arbutin and salicin	*bglR*+ *bglB*+			253/300 (84.3%)
Donor genotype	+	+	+	+
Recipient genotype	*bglB*	*bglR*	*rbs*	*bgl*
	−	+	−	−

* Strain FA31 was crossed with *P1* phage grown on strain Ca85; Rbs+ transductants were selected and scored for β-glucoside utilization.

an *ilvD-bgl* co-transduction frequency of 40 to 60% and therefore will be considered to be of the *bglR* type. Linkage between *ilvD* and *bgl* was not detectable (< 0.5%) for the other two mutants (FS20 and FS29); therefore, the mutations carried by them are probably located in an unidentified gene(s); (2) none of the amber mutations carried by strains FA35, FA37 and FA39 was found to be linked to *ilv* (< 0.5%co-transduction frequency for each); (3) the nitrosoguanidine-induced mutant FA31 was found to be a double mutant. The cross presented in Table 2 showed that in this strain there is a *bglB* mutation and a mutation capable of turning on the utilization of β-glucosides. This latter mutation is unlinked to *bglR*, since we did not find a class that does not utilize β-glucosides. It is interesting to note that this mutation has a function analogous to that of *bglR* mutations, because it turns on *bglB* expression. In fact, the phenotype of strain FA31 (utilization of arbutin, but not salicin) is identical to that of *bglR bglB* double mutants (PRASAD and SCHAEFLER 1974). This phenotype is the consequence of the presence of β-glucoside transport (coded for by the *bglC* gene) and of β-glucosidase A (the constitutively produced *bglA* gene product, which is able to hydrolize arbutin, but not salicin; see PRASAD, YOUNG and SCHAEFLER 1973).

In Table 3, we list the relevant properties of the new types of mutants found in the course of this work. Preliminary transductional experiments showed a 40 to 60% linkage of each of the six *bgl* mutations of a new type, reported in Table 3, with a *trpC* lesion. In order to locate these *trp*-linked *bgl* mutations with respect to *cysB* and *trp*, a series of three-factor crosses was performed, using P1 transduction. Table 4 shows a summary of the most relevant data. We report the data relative to only one (FA39) of the three strains carrying amber mutations, since the results obtained with the other two mutations were practically identical.

When the Cys+ phenotype was selected, the transductants inherited more frequently the *trp* than the *bgl* donor allele, thus suggesting that the latter marker is farther from *cysB*. In this set of transductions, the rarest class by far was always the one with the *trp* recipient allele and the *bgl* donor allele, which can be explained only if *trp* is the central marker. When Trp+ selection was done (Table 4), the distance between *trp* and the other two markers was found to be practically identical, and there was no rare class of transductants as expected if the *bgl* muta-

TABLE 3

New types of bgl *mutations reported in this article*

Strain designation	Selection	Mutations in the *bglBSRC* operon	Mutations not linked to the *bglBSRC* operon	β-glucosides utilized
FA31	nitrosoguanidine from PS1079	*bglB208*	*bgl-202*	arbutin
FA35	spontaneous from Ca85	—	*bgl-203* amber	arbutin and salicin
FA37	spontaneous from Ca85	—	*bgl-204* amber	arbutin and salicin
FA39	spontaneous from Ca85	—	*bgl-205 amber*	arbutin and salicin
FS20	spontaneous from PS1079	—	*bgl-206*	arbutin and salicin
FS29	spontaneous from PS1079	—	*bgl-207*	arbutin and salicin

tions lie very close to *cysB*. Therefore, we conclude that for each *bgl* mutation analyzed the order is: *cysB trp bgl*.

These mutations are probably located in a single gene for which we propose the designation *bglY*. We will show below by complementation test some evidence that supports this suggestion.

To confirm the location of *bglY* relative to *cysB* and *trp*, the *bglY207* mutation isolated in strain FS29 was further studied. Table 5 presents the results of an experiment in which the *pyrF* marker was used for selection.

bglY was also mapped with respect to the Δ(*tonB-trpAE1*) deletion. Table 6 shows that the *bglY207* mutation was transferred into strain W3110 Δ(*tonB-trpAE1*) by 73.4% of the transducing particles carrying the wild-type *trp-tonB* region; we would expect 100% if *bglY* were between *trp* and *tonB*. From this and the previous results, we conclude that the gene order is *trp tonB bglY*.

Dominance: We devised a rapid dominance test for the *bglY* mutations under study. This method was based on the use of special indicator plates that we call CEM (see MATERIALS AND METHODS), containing no nitrogenous bases, no tryptophan and a limiting amount of cystein. On these plates, strains FA31a, FA35a, FA37a, FA39a, FS20a and FS29a (each containing one of the six *bglY* mutations in a *cysB*$^-$ background) grow but show small, dark colonies, while strain KLF23/KL181 (containing the F123 episome and the *pyrD34* mutation) does not grow because pyrimidines are absent. We cross-streaked on these plates each of the *bglY* mutants and strain KLF23/KL181. After incubation, for each mutant we observed a remarkable difference in size and color of colonies between the regions on the plates where there was no mixing (small and dark colonies) and those where there was mixing (large and light colonies). This showed that the transfer of the F123 episome from strain KLF23/KL181 to *bglY* mutants abolishes the requirement for cystein and the ability to metabolize β-glucosides.

The validity of the test and of our conclusion was confirmed by performing

TABLE 4

Mapping of bgl *mutations through three-factor crosses*

Donor	Recipient	Selection	No. of transds analyzed	Unselected markers and No. transds			Classes found and no. of transductants			
				cysB	*trp*	*bgl*				
FA31 (*bgl-202*)	MI324c (*cysB⁻ trp-45*)	Cys+	192	—	79	47	*trp*+ *bgl*⁻ 42	*trp*⁻ *bgl*⁻ 5	*trp*⁻ *bgl*+ 108	*trp*+ *bgl*+ 37
FA39 (*bgl-205*)	MI324c (*cysB⁻ trp-45*)	Cys+	96	—	36	24	*trp*+ *bgl*⁻ 22	*trp*⁻ *bgl*⁻ 2	*trp*⁻ *bgl*+ 58	*trp*+ *bgl*+ 14
FS20 (*bgl-206*)	MI324c (*cysB⁻ trp-45*)	Cys+	144	—	66	24	*trp*+ *bgl*⁻ 23	*trp*⁻ *bgl*⁻ 1	*trp*⁻ *bgl*+ 77	*trp*+ *bgl*+ 43
		Trp+	288	199	—	158	*cysB*+ *bgl*⁻ 105	*cysB*⁻ *bgl*⁻ 53	*cysB*⁻ *bgl*+ 36	*cysB*+ *bgl*+ 94
FS29 (*bgl-207*)	MI324c (*cysB⁻ trp-45*)	Trp+	288	177	—	172	*cysB*+ *bgl*⁻ 108	*cysB*⁻ *bgl*⁻ 64	*cysB*⁻ *bgl*+ 47	*cysB*+ *bgl*+ 69
H677 (*trp-45*)	FS29a (*cysB⁻ bgl-207*)	Cys+	96	—	31	18	*trp*+ *bgl*⁻ 65	*trp*⁻ *bgl*⁻ 13	*trp*⁻ *bgl*+ 18	*trp*+ *bgl*+ 0

	Scheme I*			Scheme II*		
Donor genotype	+	+	—	+	—	+
Recipient genotype	cysB	trp	bgl	cysB	trp	bgl
	—	—	+	—	+	—

* All the crosses are related to Scheme I, with the exception of the one on the last line, which is related to Scheme II.

TABLE 5

Mapping of the bglY207 *mutation through a four-factor cross**

Classes found	No. of transductants
cysB+ *trp*+ *bglY*+	8/120 (6.7%)
cysB+ *trp*+ *bglY*-	2/120 (1.7%)
cysB+ *trp*- *bglY*+	31/120 (25.8%)
cysB+ *trp*- *bglY*-	0/120 (<0.9%)
cysB- *trp*+ *bglY*+	22/120 (18.3%)
cysB- *trp*+ *bglY*-	24/120 (20.0%)
cysB- *trp*- *bglY*+	32/120 (26.7%)
cysB- *trp*- *bglY*-	1/120 (0.8%)

Donor genotype	+	−	+	−
Recipient genotype	*pyrF*	*cysB*	*trp*	*bglY*
	−	+	−	+

* Strain MI324d was crossed with P1 phage grown on strain FS29a and *pyrF*+ transductants were selected. The co-transduction frequencies of *pyrF* with the other markers, calculated from the data in the table, were as follows: 65.8% with *cysB*, 46.7% with *trp* and 22.5% with *bglY*.

the dominance experiment through matings in liquid medium of the same strains mentioned above (see MATERIALS AND METHODS) before spreading the cells on plates containing all the supplements required by donor and recipient with the exception of cystein (selection against the haploid recipient) and uridine (selection against the donor). Clones from each of the matings were tested for the ability to transfer the F123 episome at high frequency and for the Bgl$^+$ or Bgl$^-$ phenotype. In each of the six cases, the presence of the episome abolished the Bgl$^+$ phenotype. The maintainance of a recessive *bglY* mutation on the chromosome of the diploid strains was demonstrated by showing that curing the episome with either acridine orange (MILLER 1972) or repeated subinocula in rich medium

TABLE 6

Mapping the bglY207 *mutation with respect to the* tonB-trpAE1 *deletion**

Classes found	No. of transductants
cysB- *bgl*-	61/192 (31.7%)
cysB+ *bgl*-	80/192 (41.7%)
cysB- *bgl*+	32/192 (16.7%)
cysB+ *bgl*+	19/192 (9.9%)

Donor genotype	−	+ +	−
Recipient genotype	*cysB*	*trp tonB*	*bgl*
	+		+

* Strain W3110Δ(*tonB-trpAE1*) was crossed with P1 phage grown on strain FS29a and Trp+ transductants were selected. All the transductants were, as predicted, sensitive to φ80vir and colicins V and B (*tonB*+ genotype). The co-transduction frequencies of the deleted region with the two external markers, calculated from the data in the table, were as follows: 48.4% with *cysB* and 73.4% with *bgl*.

yielded cultures with a high percentage of β-glucoside-utilizing cells (5 to 10%).

The conclusion from these experiments was that *bglY* mutations are recessive to the wild-type allele.

Episome mapping of bglY: The dominance experiment established that *bglY* is located within the DNA segment carried by the F123 episome, which covers *galU* but not any other marker counterclockwise to *galU*. We transferred another episome, F(col V,B *cysB*$^+$ *trp*$^+$ *tonB*$^+$) from strain W1692-YS57/Fcol into strain FS29b (*cysB*$^-$ *trp-45 bglY207*). The procedures were those used in the previous paragraph. The test on CEM plates was clear-cut since strain FS29b, due to its requirement for tryptophan, does not grow on these plates instead of forming small dark colonies. Transfer of the episome yielded diploid derivatives with a Bgl$^+$ phenotype, thus confirming the mapping data obtained from multiple-factor transductional crosses.

Complementation test: In order to gain information about the possible allelism of the various *bglY* mutations, we prepared partial diploid strains having the *bglY207* mutation on the F123 episome and each of the remaining *bglY* mutations on the chromosome. We chose the nonamber *bglY207* mutation for this test because it is most probably a point mutation since it shows a high degree of reversion (data not shown). We crossed strain DF29d (F123 *cysB*$^+$ *bglY207* / *cysB*$^-$ *bglY207*) with each of the following strains: FA31c, FA35c, FA37c, FA39c and FS20c (each containing a different *bglY* mutation in a *cysB* background). The derivatives of the latter strains carrying the episome were selected on plates that contained no cystein (selecting against the haploid recipients) or histidine (selecting against the donor). From each cross, we obtained diploids that were able to transfer the episome with high efficiency. One diploid strain from each cross was purified and tested for growth on either arbutin or salicin. All of them utilized both carbohydrates at the same rate as the haploid control. We concluded that the *bglY207* mutation does not complement any of the other *bglY* mutations. This result was strongly suggestive of the allelism of the various *bglY* mutations.

β-glucosidase B and β-glucoside transport assays: The experiments reported below were aimed at determining whether the expression of β-glucosidase B and β-glucoside uptake in *bglY* mutants is inducible, as it is in *bglR* mutants (SCHAEFLER 1967) or constitutive.

We analyzed the β-glucosidase B activity of a number of *bglY* mutants under inducing conditions in comparison to noninducing conditions. Table 7 shows that β-glucosidase B activity of all mutants analyzed was detectable only when these were grown in inducing conditions.

A qualitative estimation of uptake of β-glucosides in the *bglY* mutants under study was performed by means of the method outlined in MATERIALS AND METHODS. Colonies of each mutant turned yellow within 10 min from addition of p-nitrophenyl-β-D-glucoside when grown in inducing conditions and did not turn yellow within 4 hr when grown in noninducing conditions, showing that β-glucoside uptake in *bglY* mutants is inducible as it is in *bglR* mutants.

We devised a modification of this method, that allowed a quantitative colorimetric detection of p-nitrophenol liberated by cultures from p-nitrophenyl-β-D-

TABLE 7

*β-glucosidase B activity of induced and noninduced cells of various strains**

Strain	Specific activity	
	Induced cells	Noninduced cells
FA35 (*bglY203*)	1.85	<0.10
FS20 (*bglY206*)	1.99	<0.10
FS29 (*bglY207*)	1.59	<0.10

* Cells were grown to middle exponential phase in minimal medium containing 0.4% sodium succinate as a carbon source. Induction of β-glucosidase B was performed by adding 5×10^{-3} M β-methyl-D-glucoside.

glucoside (see MATERIALS AND METHODS). This method was applied to strain FS29 (*bglY207*) and clearly confirmed the inducibility of β-glucoside uptake (Figure 2).

Role of cyclic AMP in β-glucoside utilization: Transcription of several catabolic systems has been shown (see PASTAN and ADHYA 1976 for review) to require cyclic AMP, a compound whose synthesis in *E. coli* K12 is catalyzed by the *cya* gene product, adenyl cyclase. We tested the effects of a *cya* mutation on β-glucoside utilization in *bglR* and *bglY* mutants: strains MI385b (*bglR20 cya-283*) and FS20b (*bglY206 cya-283*) utilize arbutin and salicin only in the presence of cyclic AMP; strain FA31b (*bglY202 bglB208 cya-283*) utilizes arbutin (but not

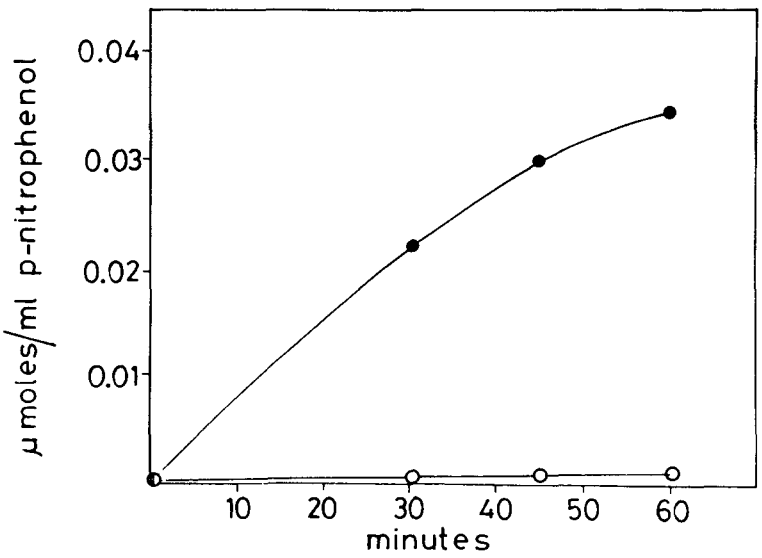

FIGURE 2.—Detection of p-nitrophenol liberated by induced (●) and noninduced (○) cultures of strain FS29 during incubation in the presence of 2×10^{-3} M p-nitrophenyl-β-D-glucoside.

salicin) only in the presence of cyclic AMP. The co-existence of *bgl* and *cya* mutations in each of these mutants was demonstrated through crosses with wild-type strains, yielding derivative classes carrying either the *bgl* mutations only or the *cya* mutation only (data not shown).

We conclude that expression of the structural genes for β-glucoside metabolism requires the activity of adenyl cyclase in both *bglR* and *bglY* mutants.

DISCUSSION

Several cryptic genetic systems that become able to perform a well-defined function as a consequence of either structural or regulatory mutations have been found in microorganisms (see RILEY and ANILIONIS 1978; DE FELICE *et al.* 1977). Apparently, these DNA segments, following the loss of ancestral phenotypic selection, have been turned off and propagated in a nonfunctional state instead of being deleted. Among these cryptic systems, the one responsible for β-glucoside utilization in *E. coli* K12 (the *bglBSRC* operon) has been most extensively studied. The *bglBSRC* operon, although not expressed, is structurally intact and can be fully activated by single regulatory mutations (PRASAD and SCHAEFLER 1974). This operon appears to be composed of two adjacent transcriptional units (*bglBS* and *bglC*) having in common the *bglR* locus, which appears to be the site from which divergent transcription of the two units procedes in β-glucoside-utilizing mutants. This is reminiscent of a number of well-known divergent transcriptional systems whose structure and function have been extensively studied in the last few years (HOFNUNG 1974; OTSUKA and ABELSON 1978; POUWELS, CUNIN and GLANDSDORFF 1974; PTASHNE *et al.* 1976; SMITH and SCHLEIF 1978).

In the present paper, we report the identification of an *E. coli* K12 locus playing a central regulatory role in *bglBSRC* gene expression. We propose *bglY* as the designation for this gene, and we show that it is located at 27 min on the *E. coli* K12 linkage map, counterclockwise to *tonB*. In the wild type, the *bglY* gene encodes a protein that is responsible *in trans* for the dormancy of the *bglBSRC* operon and therefore for the absence of activity of phospho-β-glucosidase B (coded for by *bglB*) and β-glucoside transport (coded for by *bglC*). Therefore, the expression of the metabolizing machinery is prevented by the *bglY* protein, which appears to be the only active component of the *bgl* system in the wild type (the *bglA*-encoded β-glucosidase A activity is constitutive, but inactive because of lack of transport). Our conclusions stand on the following lines of evidence: (1) three of the mutants described carry *bglY* amber mutation(s); (2) all mutations are recessive to the wild-type *bglY* allele; and (3) *bglY* mutants are capable of utilizing both arbutin and salicin, but a *bglY bglB* double mutant is capable of utilizing only arbutin.

The third line of evidence, as well as the cyclic AMP requirement for β-glucoside utilization and the inducibility of the system, are also found in mutants altered in *bglR*, the *cis*-acting regulatory site controlling the expression of the *bglBSC* genes. Therefore, it is possible that *bglY* codes for a repressor of the

bglBSRC operon, active in both the presence and absence of β-glucosides, whose recognition site would be within the *bglR* locus. Expression of the *bglBSC* genes, and therefore utilization of β-glucosides, would occur when the interaction of the repressor with the recognition site is impaired because of either *bglR* mutations (recognition site unable to bind the repressor) or *bglY* mutations (repressor absent or unable to bind at the recognition site). The recessivity of *bglY* mutations excludes the alternative hypothesis that *bglY* might code for a positive effector that is inactive in the wild type. Although consistent with our data, the repressor hypothesis must be taken cautiously until the *bglY* protein has been identified and its physical interaction with the *bglR* site demonstrated.

As outlined above, most spontaneous mutations giving rise to a *Bgl*$^-$ phenotype lie in the *cis*-acting regulatory locus *bglR* instead of in the presumably larger *bglY* locus, which is shown in the present paper to code for a protein. This is not surprising in view of the recent observation that *bglR* appears to be a site for high-frequency insertion of IS segments (A. REYNOLDS, Y. FELTON and A. WRIGHT, personal communication).

When expressed, the *bgl* system has several features that are similar to those of well-known catabolic systems. In common with these systems, *bgl* has its inducibility and cyclic AMP requirement for expression, although no information is available about the mechanism and the site of action of *bgl* inducers; furthermore, the apparent divergent transcription of the *bglBSRC* operon and the suggested positive autogenous regulatory role of the *bglS* gene product (PRASAD and SCHAEFLER 1974) are reminiscent of the *ara* system (CASADABAN 1976); finally the *bglY* protein, in our interpretation, appears to be analogous to the *lac* repressor of *lacI*^{s} mutants (MILLER 1978).

We gratefully thank: B. BACHMANN, M. LEVINTHAL, R. SOMERVILLE and C. YANOFSKY for providing us with bacterial strains; J. M. CALVO, F. CASSE DELBART, R. DIXON, J. GUARDIOLA, M. IACCARINO and C. SQUIRES for reading preliminary versions of the manuscript; and N. DELLA VOLPE and C. MIGLIACCIO for skillful technical assistance.

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Corresponding editor: H. ECHOLS