Regulation of the β-Glucoside System in *Escherchia coli* K-12

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In *Escherichia coli* wild-type cells, a mutation at the β-glucoside regulatory gene (*bglR⁺* to *bglR⁻*) leads to simultaneous expression of inducible phospho-β-glucosidase B (*bglB⁺*) and a β-glucoside-specific species of enzyme II (β-glucoside transport I [*bglC⁺*]); an additional mutation (*bglS⁺* to *bglS4*) allows these enzymes to be formed constitutively. The *bgl* alleles have been mapped in the following order: *pyrE, bglA, bglB, bglS, bglR, bglC, ilvD*. The back mutation in the regulatory allele (*bglR⁻* to *bglR⁺*) caused the cessation of the expression of the *bglB⁺*, *bglS⁺* or *bglS4*, *bglC⁺* alleles. However, a mutation in a strain with *bglB⁺*, *bglS4*, *bglR8*, *bglC⁺* alleles, at the *ini* site that lies between the *bglS4* and the *bglR8* allele, allowed the expression of the *bglS4* and *bglB⁺* alleles, but showed no affect on the expression of the *bglC⁺* allele. It is suggested that the *ini* mutation possesses a promotor-type function that in the absence of regulatory allele function (*bglR8*) renews the functioning of only the *bglS4* and *bglB⁺* alleles. The complementation studies have shown that the *bglB⁺*, *bglS⁺* or *bglS4*, *bglC⁺* alleles are expressed only in *cis* to the *bglR⁻* allele. In the constitutive strain (*bglB⁺*, *bglS4*, *bglR⁻*, *bglC⁺*), the expressed *bglS4* allele formed a soluble product that acts in *trans* over the *bglB⁺* and *bglC⁺* alleles and that appears effective only when the *bglB⁺* and the *bglC⁺* alleles are expressed in *cis* to the *bglR⁻* allele. It thus showed that the constitutive biosynthesis of phospho-β-glucosidase B and β-glucoside transport I is under positive control. Since the regulatory allele *bglR⁻* lies between the *bglS4* and the *blgC⁺* alleles, and acts in *cis*, it appears that the mutation (*bglR⁺* to *bglR⁻*) allows the initiation of transcription in one direction to express the *bglS4*, *bglB⁺* alleles and in the other to express the *bglC⁺* allele. The structural genes *bglB* and *bglC* lie adjacent to the regulatory genes *bglR* and *bglS*, and the structural genes are coordinately controlled by the regulatory genes. It is, therefore, proposed that the *bglB, bglS, bglR, bglC* genes form a *bgl* operon.

In β-glucoside-fermenting mutants of *Escherichia coli* K-12, β-glucosides such as arbutin, para-nitrophenyl β-glucoside (PNP-glu), and salicin are accumulated in a 6-phosphorylated form through the phosphoenolpyruvate-dependent phosphotransferase system (4, 12, 15). The β-glucoside-specific species of enzyme II, previously (15) designated as β-glucoside permease I, will be referred to in this paper as β-glucoside transport system I (transport I). The enzyme phospho-β-glucosidase A (*P-β-glucosidase A*) hydrolyzes phosphorylated PNP-glu and arbutin, but not phosphorylated salicin; phospho-β-glucosidase B (*P-β-glucosidase B*) hydrolyzes the same substrates as *P-β-glucosidase A* and, in addition, phosphorylated salicin. The enzymes involved in the accumulation and

hydrolysis of β-glucoside are specified by several *bgl* genes. The initial (11, 13) nomenclature of the *bgl* genes has been revised and is shown in Table 1. The location of these genes and the phenotypes of various strains carrying mutant alleles are shown in Table 3 and Fig. 1, respectively. In wild-type cells, the structural gene *bglA* is expressed and determines the constitutive biosynthesis of *P-β-glucosidase A*; the structural genes *bglB* and *bglC* are not expressed, owing to lack of a common regulatory gene expression specified by the *bglR* gene (the wild-type gene *bglR⁺*). Hence, in wild-type cells, *P-β-glucosidase B* and transport I activities are not expressed. Owing to the lack of transport I activity, wild-type cells, although possessing constitutive *P-β-glucosidase A*, are unable to catabolize PNP-glu or arbutin. A mutation, *bglR⁺* to *bglR⁻*, results in the simultaneous inducibility of the genes for both *P-β-*
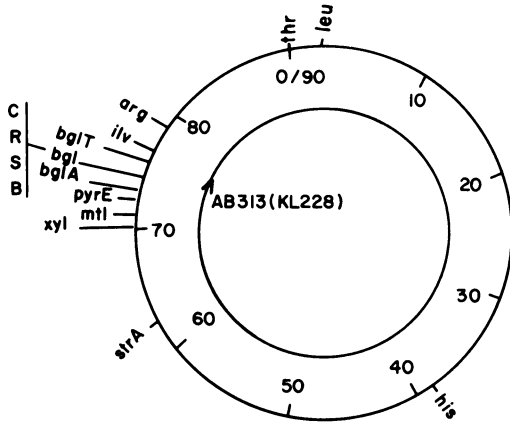
FIG. 1. *Genetic map of E. coli K-12 showing the relative position of genetic loci used in this study. Map positions were obtained from references 11, 13, 19, and the present study. Arrow head on the inner circle indicates the point of origin and direction of chromosome transfer for the Hfr strain used.*

glucosidase B ($bglB^+$) and transport I ($bglC^+$). The mutant strain is then able to synthesize and catabolize phosphorylated arbutin, PNP-glu, and salicin. In this inducible strain, an additional mutation at the $bglS$ site ($bglS^+$ to $bglS4$) causes constitutive biosynthesis of P-β-glucosidase B and transport I. The function of the $bglS4$ allele depends upon the continued expression of the $bglR^-$ allele; if the $bglR^-$ allele is back-mutated to the $bglR^+$ allele, the inducible or constitutive P-β-glucosidase B and transport I activities are not detected. It, therefore, appears that there are two regulatory genes, $bglR^+$ and $bglS^+$, which control the expression of the $bglB^+$ and $bglC^+$ genes.

In this paper we discuss the genetic mapping of the $bglC$ gene in relation to other bgl loci and the $ilvD$ gene. We also discuss the effect of the $bglR$ function on the expression of the $bglG$, $bglS$, and $bglC$ alleles as indicated from dominance tests. These results, together with earlier data (11, 13; I. Prasad and S. Schaefler, abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, G127, p. 51) form the basis of our present interpretation of the bgl system as outlined above.

Previous investigation (14) has shown that in microorganisms there are two β-glucoside transport systems. Transport I which has high affinities for arbutin and salicin, but not for cellobiose, is found in *E. coli* mutant cells. The transport system II, previously designated as permease II, has high affinity for cellobiose, but not for arbutin or salicin. In *E. coli*, the $bglC$ locus which determines the β-glucoside-specific enzyme II of the phosphoenolpyruvate-depend-

ent phosphotransferase system (transport I) lies between the bgl cluster and the $ilvD$ gene. The transport system II is found in *Citrobacter* and *Aerobacter aerogenes* and is possibly identical with the adenosine 5'-triphosphate-dependent cellobiose phosphotransferase as recently described by Palmer et al. (10). In this paper, we also report the presence of a third β-glucoside constitutive transport system (transport III) which has high affinities for arbutin and salicin and is found in an *E. coli* K-12 mutant strain. Transport III is specified by the $bglD$ gene which is not linked with the $ilvD$ gene or the bgl cluster (S. Schaefler and I. Prasad, Int. Congr. Biochem., 9th, 1973, abstr.). The regulation of the transport III system will be reported elsewhere.

MATERIALS AND METHODS

Nomenclature and strains. Symbols assigned for various bgl genes are in accordance with the nomenclature suggested by Demerec et al. (2). In previous papers (11, 13) Prasad and Schaefler, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1972, G127, p. 51) the designation of bgl genes were not consistent with their phenotypic nomenclature; therefore, currently we have revised the nomenclature of the bgl genes. Table 1 shows both the old and new bgl symbols corresponding to their phenotypes. Independently isolated mutants (Table 3) have been designated by a different Arabic number ($bglR1$, $bglR2$, and so on). Not all of these mutants isolated for the same locus were tested to see whether or not they are identical. All the bacterial strains are derivatives of *E. coli* K-12 and they are listed in Table 2. The bacteriophage used for transduction was P1. Table 3 shows the bgl genotypes and phenotypes of the mutants isolated from different strains.

TABLE 1. *Nomenclature of the bgl genes with their phenotypes*

Previously assigned bgl loci	Newly assigned bgl loci	Phenotype (enzyme activities)
bglD	bglA	Structural gene for P-β-glucosidase A
bglA	bglB	Structural gene for P-β-glucosidase B
bglB	bglC	Structural gene for β-glucoside transport I
	bglR	Regulatory gene or site for β-glucoside transport I and P-β-glucosidase B
bglC	bglS	Regulatory gene or site for β-glucoside transport I and P-β-glucosidase B
bglE	bglT	A site for the hyper production of P-β-glucosidase A
	bglD	Structural gene for β-glucoside transport III

TABLE 2. *Bacterial strains*

Strains[a]	Sex	Genotypes[b]	Reference or source
WT *E. coli* K-12 SP2, SP3	F[+]	Prototrophs, *str*[+]	(12, 13)
SP5, SP6, SP19 SP21, SP31, SP33	F^{−}	Prototrophs, *str*[+]	This paper (12, 13)
SP7, SP9, SP12 SP23, AB1450	F^{−}	*arg, ilv, thi, his, xyl, mtl mal, str*	(13)
SP10, SP15 SP24, SP30, SP32	F^{−}	*arg, met, thi, his, xyl, mtl mal, str*	This paper
SP11, SP13	F^{−}	*arg, met, thi, his, pyrE, mtl, str*	(11)
SP27	Hfr	*leu, str*[+]	This paper (derived from KL228)
AB1458	F^{−}	*pyrE, arg, his, pro, leu, thr, thi, xyl, str*	W. Maas
KL16-99	Hfr	*recA1, thi*	Brooks Low
CGSC-4502	Hfr	*pyrE41, metB1, tna 22*	B. Bachman
KL228	Hfr	*leu, str*[+]	Brooks Low
JC1993	F^{−}	*arg, leu, his, met, str, xyl, mtl, mal, recA*	Brooks Low
SPF2	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[+]*,C*[+]*)/JC1993*	This paper
SPF3	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[+]*,C*[+]*)/SP13-recA1*	This paper
SPF4	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[+]*,C*[+]*)/SP30-recA1*	This paper
SPF5	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[+]*,C*[+]*)/SP11-recA1*	This paper
SPF6	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[−]*,C*[+]*)/SP30-recA1*	This paper
SPF10	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[−]*,C*[+]*)/SP13-recA1*	This paper
SPF11	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[−]*,C*[+]*)/SP12-recA1*	This paper
SPF12	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[+]*,C*[+]*)/SP9-recA1*	This paper
SPF13	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[−]*,C*[+]*)/SP32-recA1*	This paper
SPF14	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[+]*,C*[+]*)/SP32-recA1*	This paper
SPF15	F'	*bgl (A*[+]*,B*[+]*,S*[+]*,R*[−]*,C*[+]*)/SP23-recA1*	This paper

[a] WT, wild-type strain; SP, mutant strain in the β-glucoside system, obtained in this laboratory SPF, merodiploid strains possessing exogenates with different *bgl* alleles, constructed in this laboratory.

[b] These symbols have been described in reference (19).

Construction of strains. Isolation procedure for the strains SP2, SP3, SP5, SP6, SP7, and SP9 (Table 3) has been discussed in previous papers (11, 13). Strain SP10 was prepared by co-transducing the *bglR1* and the *bglS4* allele with the *ilvD*[+] marker from the strain SP3 into strain SP9. Among *ilv*[+] colonies, we selected those that did not grow on arbutin or salicin medium nor show P-β-glucosidase A and P-β-glucosidase B activities in intact cells or in cell extracts. The introduced *bglS4* allele in SP10 was verified in two ways: (i) SP10 was back-mutated to grow on arbutin medium, thus restoring the P-β-glucosidase A activity. Constitutive transport I activity was detected by the addition of PNP-glu. (ii) A P1 lysate was prepared with SP10 as donor, and the *bglR1* and *bglS4* alleles were co-transduced with *ilvD*[+] marker into strain AB1450. Thirty-five percent of the *ilv*[+] transductants showed constitutive transport I and P-β-glucosidase B activities, thus confirming the presence of *bglS4* allele in strain SP10. Strain SP11 was constructed by conjugation introducing the *pyrE* allele from the strain CGSC-4502 into strain SP10 and selecting for *xyl*[+] colonies. Among the *xyl*[+] colonies, one which acquired the *pyrE* allele and retained the *bgl* alleles of strain SP10 was selected.

Strains SP12 and SP13 were constructed by transducing the *bglA*[+] allele from the wild-type strain into strains SP9 and SP11 and selecting for Arb[+] colonies. Strain SP15 was constructed by co-transducing the *bglR1* and *bglS4* alleles with an *ilvD*[+] marker from strain SP3 into strain AB1450. Colonies were selected for the utilization of arbutin and salicin which showed constitutive P-β-glucosidase B and transport I activities. Strain SP27 was constructed by transducing the *bglR1* allele from the strain SP2 into strain KL228. The isolation procedures and detailed description of transport I-defective mutants (SP19, SP21, SP23, SP24, SP30) and *ini* mutants (SP31, SP32) are discussed in the results. The transport III mutant (SP33) is mentioned in Schaefler and Prasad (Int. Congr. Biochem., 9th, 1973, abstr.) and will be discussed in detail elsewhere.

recA strains. Hfr strain KL 16-99 (7) with genotype *his*[+], *str*[+], *recA*[−], which transfers the *recA*[−] allele as an early marker, was mated with the F^{−} strains SP9, SP11, SP12, SP13, SP30, SP32, and SP23 possessing *his*[−], Str[R] mutations. From the mating mixture, the recombinant *his*[+], Str[R] colonies were isolated. The recombinants that were mitomycin C sensitive at a concentration of 0.8 μg/ml and ultravio-

TABLE 3. *Genotypes corresponding to the phenotypes of the β-glucoside system*

Strain[a]	Genotype[b]						Enzyme[c]				Mutant[d]	Fermentation[e]	
							P-β-glucosidase		Transport				
	blgA	bglB	bglC	bglD	bglR	bglS	A	B	I	III	bgl alleles	Arbutin	Salicin
Wild type	+	+	+	+	+	+	+c	−	−	−	Wild type	−	−
SP2	+	+	+	+	−	+	+c	+I	+I	−	R1	+	+
SP3	+	+	+	+	−	+c	+c	+c	+c	−	R1,S4	+	+
SP5	+	−	+	+	−	+	+c	−	+I	−	B5,R1	+	
SP6	+	−	+	+	−	+c	+c	−	+c	−	B12,R1,S4	+	
SP7	+	+	+	+	−	+	+c	+I	+I	−	R2	+	+
SP9	−	−	+	+	−	+	−	−	−	−	A7,B13,R2	−	−
SP10	−	−	+	+	−	+c	−	−	+c	−	A7,B13,R1,S4	−	−
SP11	−	−	+	+	−	+c	−	−	+c	−	A7,B13,R1,S4	−	−
SP12	+	−	+	+	−	+	+c	−	+I	−	B13,R2	+	−
SP13	+	−	+	+	−	+c	+c	−	+c	−	B13,R1,S4	+	−
SP15	+	+	+	+	−	+c	+c	+c	+c	−	R1,S4	+	+
SP19	+	+	−	+	−	+	+c	+I	−	−	R1,C9	−	−
SP21	+	+	+	+	+	+c	+c	−	−	−	R6,S4	−	−
SP23	+	+	−	+	−	+	+c	+I	−	−	C11,R2	−	−
SP24	+	+	−	+	−	+	+c	+I	−	−	C11,R2	−	−
SP27	+	+	+	+	−	+	+c	+I	+	−	R1	+	+
SP30	+	+	+	+	+	+c	+c	−	−	−	R10,S4	−	−
SP31	+	+	+	+	+	+c	+c	+c	−	−	R8,ini,S4	−	−
SP32	+	+	+	+	+	+c	+c	+c	−	−	R8,ini,S4	−	−
SP33	+	+	+	−	+	+	+c	−	−	−c	R3,D14	+	−

[a] Some of the SP strains were described in previous papers under the following nomenclature: SP2 = β-gl⁺ or prototroph (*bglD⁺, bglA⁺, bglC⁺, bglB⁺, bglE⁺*)/2. SP3 = β-gl⁺c or prototroph (*bglD⁺, bglA⁺, bglCᶜ, bglB⁺, bglE⁺*)/3. SP5 = β-gl⁺ sal⁻ or prototroph (*bglD⁺, bglA⁻, bglC⁺, bglB⁺, bglE⁺*)/4. SP6 = β-gl⁺ sal⁻c or prototroph (*bglD⁺, bglA⁺, bglCᶜ, bglB⁺, bglE⁺*)/7. SP7 = AB1450 β-gl⁺ or AB1450 (*bglD⁺, bglA⁺, bglC⁺, bglB⁺, bglE⁺*)/2. SP9 = AB1450 (*bglD⁻, bglA⁻, bglC⁺, bglB⁺, bglE⁺*)/5. Strains SP10, SP11, SP12, SP13, SP15, SP19, SP21, SP23, SP24, SP27, SP30, SP31, and SP33 have been discussed in Materials and Methods.

[b] The symbols *bglA, bglB, bglC, bglD, bglR, bglS* have been described in Table 1; +ᶜ, constitutive allele for P-β-glucosidase B and β-glucoside transport I; +, bgl allele is present; −, the activity of bgl allele is not detected.

[c] c, Constitutive formation; I, inducible formation; −, no activity detected.

[d] Strain possessing mutant bgl allele and the isolate number for each mutant allele.

[e] +, Growth; −, no detectable growth.

let sensitive as tested by the method of Clark et al. (1) were selected as *recA* strains. The *recA⁻* characteristic of the recombinant strains was further confirmed by their inability to yield recombinants from conjugation and transduction.

Media. The various media used in this investigation have been described in a previous paper (12).

Nutrient medium A, containing arbutin, salicin, or xylose as carbon source, has been referred to arbutin, salicin, or xylose medium. Colonies capable of growing on arbutin, salicin, or xylose medium have been referred to Arb⁺, Sal⁺, Xyl⁺, and similarly the colonies incapable of growing on these media have been referred to Arb⁻, Sal⁻, Xyl⁻.

Mutagenesis. The treatment with nitrous acid was as follows: 5 ml of overnight culture was washed with 5 ml of 0.1 M acetate buffer, pH 4.6. Cells were suspended in 0.3 ml of freshly prepared nitrous acid solution (16) for 10 min at 37 C, and then the reaction was stopped by addition of 5 ml of M63 buffer which was prepared by the method of Schwartz and Beckwith (16). The cells were centrifuged and resuspended in 10 ml of LB broth and grown for 6 h. The culture was diluted and plated on selective medium to score for mutants. The mutagenesis with ethyl methane sulfonate was performed as reported earlier (11). Arb⁻ Sal⁻ colonies were isolated as follows: mutagenized cells of an Arb⁺ Sal⁺ culture were plated on salicin medium and incubated for 24 h. Under a dissecting microscope, minute colonies were isolated and subcultured on solid LB medium and then were replicated on arbutin and salicin solid media. Those colonies that failed to grow on arbutin or salicin medium but grew on the glucose medium were purified by single-colony isolation. P-β-glucosidase A, P-β-glucosidase B, and transport activities were tested in induced and noninduced cells.

Enzyme assays. P-β-glucosidase A activity in intact cells or toluene-treated cells was determined as described in the previous paper (12). P-β-glucosidase B activity was tested as follows: cells were grown in liquid succinate medium to an optical density of 0.38 at 590 nm. For whole cells, 2 ml of culture was centrifuged, and cells were washed twice with 0.75 M phosphate buffer at pH 7.6 and resuspended in 0.3 ml of phosphate buffer. Salicin (0.1 ml) (0.2 M) and 0.2 ml of water were added; the mixture was incubated for 20 min at 37 C. The reaction was stopped by addition of 0.5 ml of 2 M Na_2CO_3. Cell extracts were prepared by addition of 0.1 ml of lysozyme (2 mg/ml) to washed cells suspended in 0.3 ml of 10^{-4} M ethylenediaminetetraacitic acid (dissolved in 0.75 M tris (hydroxymethyl)-aminomethane at pH 8.0) and incubated for 20 min at 37 C. Then, 0.1 ml of salicin (0.2 M), 0.1 ml of Glu-6-PO₄ (3×10^{-4} M), and 0.1 ml of phosphatase-transphosphorylase (gl-phosphotransferase) (15) were added in the mixture and incubated for 20 min at 37 C (12). The reaction was stopped by addition of 0.5 ml of 2 M Na_2CO_3. Then, 0.5 ml of 0.6% 4-amino-antipyrine was added to each reaction mixture prepared with whole cells or cell extract. After 15 min, 0.5 ml of 4% K_3 (CN)₆ Fe was added to each mixture. Cells or cell extracts possessing P-β-glucosidase B activity showed a red color that was measured spectrophotometrically (Gilford spectrophotometer) after 5 min at 509 nm (saliginine reaction) (12).

Transport activities. For screening purposes the following methods were used to distinguish between constitutive and inducible transport activities: *E. coli* K-12 cells possess a constitutive P-β-glucosidase A activity which hydrolyzes phosphorylated PNP-glu and liberates p-nitrophenol; it can be expressed in whole cells only in the presence of an active transport system. Approximately 50 colonies were grown on succinate solid medium, and 1 ml of 4×10^{-2} M PNP-glu solution was spread over the colonies. Colonies made up of cells possessing constitutive transport activities accumulated PNL-glu and turned yellow, whereas colonies of cells with inducible transport activities did not change in color. In order to confirm the inducibility of the latter type, cells were grown in liquid succinate medium with β-methyl glucoside (5 $\times 10^{-3}$ M) as inducer (12). Induced cells were washed with phosphate buffer (pH 6.8) and then exposed to PNP-glu (4×10^{-2} M). If the cells possessed P-β-glucosidase B activity, the constitutive or inducible transport activities were distinguished by testing for the saliginine reaction. The accumulation of ¹⁴C-labeled β-glucoside was measured by the method reported earlier (11).

Genetic mapping. The procedure for transduction and conjugation have been described in a previous paper (13).

Isolation of merodiploids. Hfr strain KL228 (8) is a derivative of Hfr strain AB313 which transfers the *bgl, pyrE, xyl⁺* genes, in this order, as early markers and gives rise to a stable F′ factor at a high frequency. In these studies all the recipient strains possessed a *recA⁻* allele, so that integration of the exogenote into the chromosome was minimized (6). Merodiploid strains listed in Tables 2 and 7 were constructed as follows: merodiploid strain SPF2 was isolated by mating strain SP27 with recipient strain JC1993. The merodiploid strains SPF3, SPF4, SPF5, SPF12, and SPF14 were isolated by mating SP27 with SP13 *recA*, SP30 *recA*, SP11 *recA*, SP9 *recA*, and SP32 *recA*, respectively. The merodiploid strains SPF6, SPF10, SPF11, and SPF13 were isolated by mating KL228 with SP30 *recA*, SP13 *recA*, and SP12 *recA*, and SP32 *recA*, respectively. In each instance donor and recipient cells were mated in a ratio of 1:10, respectively, for 1 h. The mating mixture was diluted 1:10 with A-N buffer. The mating was interrupted with a Vortex mixer. The interrupted mixture was again diluted 1:5 with buffer, and 0.1-ml samples were plated on xylose medium containing streptomycin (100 µg/ml). Upon incubation, *xyl⁺*, Str^R merodiploid strains were isolated. The presence of the exogenote was confirmed by eliminating it from the suspected merodiploid strains with acridine orange treatment and recovering the *xyl⁻* marker of the endogenote. Furthermore, the exogenote was tested for its ability to transfer *xyl⁺*, *pyrE⁺*, and *bgl* markers into strain AB1458 without transferring *his⁺* or *pro⁺* markers.

Elimination of exogenote from the merodiploid strain. A loopful of an overnight culture of the merodiploid strain was inoculated into 1 ml of LB broth, pH 7.6, containing 50 µg of acridine orange. Cells were grown for 6 h, diluted, and plated on LB solid medium. Colonies obtained were replicated on selective media to observe the elimination of F′ by detecting the markers of the endogenote.

RESULTS

Characterization of mutations involved in the regulation of P-β-glucosidase B and transport I. Previous work (12, 13) indicated that a single-step mutation in wild-type cells causes the expression of inducible P-β-glucosi-

dase B and transport I. This mutation occurs at the *bglR* gene, previously designated as the *bglB* gene. In previous studies, this mutation was considered either located at the structural gene for transport I or else in a separate locus (regulatory site). In order to distinguish between these two possibilities various types of mutants were isolated and characterized.

Transport I-defective mutants. These mutants resulted from mutation at two different genetic loci, one of which apparently represented the structural gene for transport I (*bglC*) and the other of which represented a common regulatory gene (*bglR*) for both *bglB+* and *bglC+*.

bglC⁻ mutants. Strain SP19 (*bglR1*, *bglC9*) was selected by the following procedure: strain SP2 mutagenized with nitrous acid, and Arb⁻ Sal⁻ colonies were isolated. The colonies were grown in the presence of inducer, and the mutants which showed P-β-glucosidase B activity with cell extracts, but not with intact cells, were selected. It was considered that this mutant lacked transport I activity, owing to a mutation at the structural gene for transport I (*bglC9*), and retained the regulatory allele (*bglR1*) that allowed the expression of inducible P-β-glucosidase B activity (Fig. 2). The presence of *bglR1* and *bglC9* alleles in strain SP19 was confirmed by the fact that P1 lysate prepared with wild-type strain (*bglR+*, *bglC+*) was able to transduce the *bglC+* gene into strain SP19 to yield Sal⁺ Arb⁺ (*bglR1*, *bglC+*) recombinants possessing inducible P-β-glucosidase B and transport I activities. Strain SP23 (*bglR2*, *bglC11*) was obtained by ethyl methane sulfonate treatment of the strain SP7, following the same procedure as the construction of strain SP19. Strain SP24 (*bglR2*, *bglC11*) was constructed by transducing the *ilvD+* marker from wild type to strain SP23.

bglR+ revertants. Strain SP21 (*bglS4*, *bglR6*, *bglC+*) was constructed by mutagenizing the strain SP3 with nitrous acid, and the strain SP30 (*bglS4*, *bglR10*, *bglC+*) was constructed by mutagenizing strain SP15 with ethyl methane sulfonate. In both instances Arb⁻, Sal⁻ colonies were isolated. The criteria of selection was, upon induction with β-methyl glucoside (5 × 10⁻³ M), lack of transport I activity with intact cells (Fig. 2) and P-β-glucosidase B activity with intact cells and cell extracts. It was thought that a mutation *bglR⁻* to *bglR+* blocked the expression of both P-β-glucosidase B and transport I activities. To test this hypothesis transductional crosses were performed. Strains SP21 and SP30 were crossed individ-

ually with strains wild-type (*bglR+*, *bglC+*) and SP19 (*bglR1*, *bglC6*), and selection was made for Sal⁺ recombinants. In each instance the cross with wild type did not yield any such recombinant, but with strain SP19 it yielded Sal⁺ recombinants, suggesting that strains SP21 and SP30 both possess *bglR+* and *bglC+* alleles. Furthermore, the preservation of the *bglS4* allele in strains SP21 and SP30 was formed by co-transduction of the *bglS4* allele with the *bglB+* gene into strain SP5 by selecting for Sal⁺ colonies. Some of the selected colonies possessed constitutive P-β-glucosidase B and transport I activities.

Initiator (ini) mutation for the bglB and bglS genes. Mutant strain SP31 was selected among Arb⁻ Sal⁻ colonies isolated from ethyl methane sulfonate-treated strain SP3. The selected colony showed constitutive P-β-glucosidase B activity with cell extracts, but not with intact cells, and failed to show transport I activities with PNP-glu assay and uptake determinations (Fig. 2) with induced and noninduced culture. In strain SP31, the level of P-β-glucosidase B activity was ten times lower than that of the parental strain (Table 4). Upon induction with β-methyl glucoside, the strain SP31 showed threefold increase in the level of P-β-glucosidase B activity. To determine the mutation site in strain SP31, phage grown on the wild-type strain (*bglR+*, *bglC+*) and on strain SP19 (*bglR1*, *bglC9*) were used to transduce strain SP31. The selection was for Sal⁺ colonies. The first cross did not yield any Sal⁺ recombinants, whereas the latter did, suggesting that the SP31 mutant apparently lacked regulatory gene expression (*bglR8*). Furthermore, in strain SP31, the presence of mutant
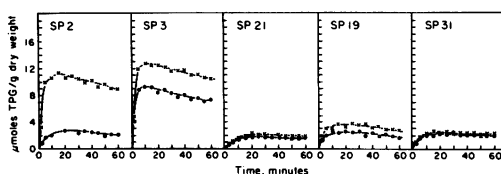

Fig. 2. *Uptake of [¹⁴C]thiophenyl β-glucoside by non induced cells and cells induced by β-methyl glucoside. Cultures of inducible (SP2), constitutive (SP3), transport I-defective (SP19, SP21), and ini (SP31) strains were grown in medium A (noninduced) and medium A with β-methyl glucoside (5 × 10⁻³M). Washed cells (400 g [dry weight]/ml) were suspended in buffer with [¹⁴C]thiophenyl β-glucoside, specific activity 4 mCi/mmol, at a concentration of 2 × 10⁻⁴ M (20,000 counts/min). The uptake was determined at 28 C. At different times the accumulated triophenyl β-glucoside was determined. Symbols: ×, induced cells; O, noinduced cells.*

TABLE 4. *P-β-glucosidase B activity detected in different mutant strains[a]*

Strain	Intact cells		Cell extracts[b]	
	Induced	Non-induced	Induced	Non-induced
SP2	20	–	15	–
SP3	20	11.5	15	5
SP27	19	–	13	–
SP31	–	–	1.8	0.5
SP32	–	–	1.6	0.5

[a] Cultures grown in succinate medium were washed with phosphate buffer (pH 7.6 at 0.75 M) and tested for P-β-glucosidase B activity. Activity is expressed in nanomoles per minute per milligram (dry weight) of cells. Enzyme was induced by β-methyl glucoside (5 × 10^{-3} M).

[b] Cell extracts: procedure is described in Materials and Methods. (–), No activity.

allele bglR8 was confirmed by co-transducing it with the ilvD$^+$ gene into strain SP7 at a frequency of 33%. The recombinants (bglB$^+$, bglS$^+$, bglR8, bglC$^+$) lacked P-β-glucosidase B activity. Therefore, it appeared that strain SP31 resulted from mutation at two separate loci, one mutation at the regulatory locus (bglR) and the other mutation at ini that allowed the expression of constitutive P-β-glucosidase B activity without simultaneous expression of the bglR gene. The ini mutation has no effect on the expression of the bglC$^+$ gene. From strain SP31, the ini mutation was co-transduced at a low frequency with the bgl cluster into strain SP7, by selecting for ilv$^+$ colonies. In this way strain SP32 was constructed.

Mapping of the ini mutation. Table 5 shows the mapping data. P1 lysate prepared with the strain SP32 was used to transduce the bglB$^+$ allele into strain SP5 by selecting for Sal$^+$ colonies. Out of 30 Sal$^+$ recombinants, 20 showed constitutive P-β-glucosidase B and transport I activities [bgl (B$^+$, S4, ini, R$^-$, C$^+$)], whereas the ten remaining recombinants showed inducible P-β-glucosidase B and transport I activities [bgl(B$^+$, S$^+$, R$^-$, C$^+$)]. If the mutation ini had been located within or close to the bglB$^+$ locus, all Sal$^+$ recombinants would have possessed constitutive P-β-glucosidase B activity. Therefore, it is likely that the ini mutation lies very close to the bglS4 allele. Twenty Sal$^+$ recombinants possessing constitutive P-β-glucosidase B activity also showed constitutive transport I activity, indicating that they had expressed bglS4 allele. Table 7 shows that the bglS4 allele is expressed cis to the bglR$^-$ allele which lies to the right of the bglS4

allele. It is, therefore, assumed that the ini mutation which expresses the bglS4 allele in absence of the bglR gene function lies to the right of the bglS4 allele. Experiment 2 of Table 5 shows that the recipient strain SP33 possesses bglD14 allele that expresses the constitutive transport III activity, but not the bglB$^+$ and bglC$^+$ alleles. The ini mutation was transduced into strain SP33 by selecting Sal$^+$ colonies. All selected Sal$^+$ colonies possessed constitutive P-β-glucosidase B activity, indicating that the ini mutation is probably located very close to the bglS4 allele. In experiment 3, the cross was of the same type as shown in experiment 1 except that the donor was SP31, but the results obtained were consistent with those of experiment 1.

Mapping of the bglC gene. By conjugation, Hfr strain KL228 possessing the wild-type bgl allele (bglR$^+$, bglC$^+$) was mated with the recipient strain SP23 (bglR$^-$, bglC$^-$), and selection was made for xyl$^+$ Str^R colonies. Among xyl$^+$, Str^R recombinants, approximately 60% were Arb$^+$ Sal$^+$ (bglR$^-$, bglC$^+$). The result suggested that the bglC gene was present but unexpressed and was located near the origin of Hfr strain KL228 (Fig. 1). Previously, the bgl cluster was mapped near the origin of Hfr strain AB313 (11, 13).

A transduction experiment performed with phage P1 showed that the bglS, bglR, and bglC genes were co-transduced with the pyrE marker at a frequency of 1.5, 1.5, and 1.7%, respectively (13). The genes bglS, bglR, and bglC were co-transduced with the ilvD$^+$ marker at a frequency of 33, 35, and 35%, respectively (13). It, therefore, appears that the bglR and bglC genes are situated very close to each other on the E. coli linkage map. Three-point crosses were performed for finer mapping, and the results are given in Table 6. The analysis of the crosses described in Table 6 suggests the following gene order: pyrE, bglA, bglB, bglS, bglR, bglC, ilvD. If this gene order is correct, the cross between bglR$^+$, bglC$^+$ and bglR$^-$, bglC$^-$ (experiment 1) requires two crossovers to yield recombinants of the genotype bglR$^-$, bglC$^+$ upon selection for ilv$^+$ colonies, whereas the reciprocal cross (experiment 2) requires four crossovers to yield the same type of recombinants for the same selection. The results of experiment 1 yielding 35% recombinants and experiment 2 yielding 2% recombinants are in agreement with the suggested gene order. In experiment 3 the cross performed was of the same type as shown in experiment 1, except that the donor possessed the bglR$^+$ allele. In this cross, 37% of the selected ilv$^+$ colonies were recombinants of the

TABLE 5. *Mapping by transduction of ini mutation*[a]

Expt no.	Crosses	Selected marker/ no.	Unselected recombinant type/no. scored
1	Donor SP32 $bgl(B^+,S4,ini,R8,C^+)$ Recipient SP5 $bgl(B5,S^+,R1,C^+)$	Sal⁺/30	B^+ $S4$ ini $R8$ C^+ / $B5$ S^+ $R1$ C^+ a. $B^+,S^+,R1,C^+$/10 B^+ $S4$ ini $R8$ C^+ / $B5$ S^+ $R1$ C^+ b. $B^+,S4,ini,R8,C^+$/20
2	Donor SP32 $bgl(B^+,S4,ini,R8,C^+;D^+)$ Recipient SP33 $bgl(B^+,S^+,R3,C^+;D14)$	Sal⁺/20	B^+ $S4$ ini $R8$ C^+ D^+ / B^+ S^+ $R3$ C^+ $\overline{D14}$ a. $B^+,S4,ini,R3,C^+;D14$/20 B^+ $S4$ ini $R8$ C^+ D^+ / B^+ S^+ $R3$ C^+ $\overline{D14}$ b. $B^+,S^+,R3,C^+;D14$/0
3	Donor SP31 $bgl(B^+,S4,ini,R8,C^+)$ Recipient SP5 $bgl(B5,S^+,R1,C^+)$	Sal⁺/17	B^+ $S4$ ini $R8$ C^+ / $B5$ S^+ $R1$ C^+ a. $B^+,S^+,R1,C^+$/5 B^+ $S4$ ini $R8$ C^+ / $B5$ S^+ $R1$ C^+ b. $B^+,S4,ini,R1,C^+$/12

[a] Salicin-fermenting colonies isolated from each experiment were individually tested for the presence of inducible and constitutive P-β-glucosidase B and β-glucoside transport I activities with intact cells and inducible and constitutive P-β-glucosidase B activity with cell extracts.

genotype $bglR^-$, $bglC^+$. In experiment 4, Sal⁺ colonies were selected ($bglB^+$), and 99.5% of the recombinants were inducible ($bglS^+$) and 0.5% constitutive ($bglS4$). If the suggested gene order is correct, then experiment 4 requires four crossovers to yield recombinants of the genotype $bglS4$, $bglR^-$, $bglC^+$ and two crossovers to yield recombinants of the genotype $bglS^+$, $bglR^-$, $bglC^+$; this is consistent with the recombination frequency given in Table 6.

Complementation analysis. Regulation of the biosynthesis of P-β-glucosidase B and transport I was analyzed by the construction of merodiploids as shown in Table 7. In strain SPF2, the exogenote derived from the strain SP27 possesses a functional $bglR^-$ allele capable of expressing the $bglC^+$ and $bglB^+$ genes which determine inducible P-β-glucosidase B and transport I, whereas the endogenote possesses the $bglR^+$ allele which blocks the expres-

sion of $bglB^+$ and $bglC^+$ genes. The merodiploid strain showed growth on arbutin and salicin medium, suggesting that the wild-type $bglR^+$ allele is recessive and apparently does not form soluble repressor molecules. In this merodiploid strain the level of inducible P-β-glucosidase B activity was similar to that detected with the strain SP27 (Table 4). In strain SPF12, the exogenote possesses the $bglA^+$ and $bglB^+$ alleles, whereas the endogenote possesses $bglA^-$ and $bglB^-$ alleles. The merodiploid strain showed only P-β-glucosidase B activity, but not P-β-glucosidase A. The presence of active $bglA^+$ allele on the exogenote was confirmed by integrating the exogenote into the chromosome of a $bglA^-$ mutant strain (SP9) and determining P-β-glucosidase A activity. The merodiploid strain showed that the $bglB^-$ allele of the endogenote was recessive, and the expression of the $bglA^+$ allele on the exogenote requires

TABLE 6. *The mapping by transduction of bglC locus*

Expt no.	Donor	Recipient	Selected recombinant types[a] (no. of colonies analyzed)	Unselected recombinant types[b] [%]	Crossover regions[c]
1	Wild-type $bgl(B^+,S^+,R^+,C^+)$	SP23 $bgl(B^+,S^+,R^-,C^-)iluD^-$	Ilv$^+$ (300)	$bgl(B^+,S^+,R^-,C^+)$ [35]	I, II
2	SP24 $bgl(B^+,S^+,R^-,C^-)$	AB1450 $bgl(B^+,S^+,R^+,C^+)iluD^-$	Ilv$^+$ (600)	$bgl(B^+,S^+,R^-,C^+)$ [2]	I, II, III, IV
3	SP21 $bgl(B^+,S4,R^+,C^+)$	SP23 $bgl(D^+,S^+,R^-,C^-)iluD^-$	Ilv$^+$ (300)	$bgl(B^+,S^+,R^-,C^+)$ [36]	I, II
4	SP21 $bgl(B^+,S4,R^+,C^+)$	SP19 $bgl(B^+,S^+,R^-,C2^-)$	Sal$^+$ (600)	a. $bgl(B^+,S4,R^-,C^+)$ [0.5] b. $bgl(B^+,S^+,R^-,C^+)$ [99.5]	II, III, IV, V II, III

[a] Ilv$^+$, Isoleucine-valine-independent growing colonies; Sal$^+$, colony growing on medium containing salicin as carbon source.

[b] Unselected recombinants were tested by their abilities to grow on medium containing arbutin or salicin as carbon source and to express the constitutive or inducible P-β-glucosidase B and β-glucoside transport I activities.

[c] Crossover regions in the suggested map:

```
          V     IV    III    II    I
       pyrE  blgA  bglB  bglS  bglR  bglC  iluD
```
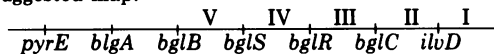
another gene function that was not present on the exogenote (11).

In strain SPF11, the exogenote lacks the regulatory gene function ($bglR^+$) and is incapable of expressing P-β-glucosidase B ($bglB^+$) and transport I ($bglC^+$) activities, whereas the endogenote possessing the $bglB^-$, $bglS^+$, $bglR^-$, $bglc^+$ allele is capable of expressing transport I but not P-β-glucosidase activity. The strain grew on arbutin medium, but not on salicin medium, and did not show P-β-glucosidase B activity. This experiment suggests that the $bglR^-$ allele does not act in *trans* to allow the $bglB^+$ gene of the exogenote to form P-βglucosidase B. However, the experiments with strains SPF2 and SPF12 show that $bglB^+$ allele is expressed in *cis* to $bglR^-$ allele. In strain SPF10 the situation was the same as in strain SPF11, except that the endogenote possessed the $bglS4$ allele *cis* to the $bglR^-$ allele. Cells failed to show P-β-glucosidase B activity.

In strain SPF3 (F' $bglB^+$, $bglS^+$, $bglR^-$, $bglC^+$/$bglB^-$, $bglS4$, $bglR^-$, $bglC^+$) the bgl alleles were the same as in the strain SPF12, except that the endogenote possessed the $bglS4$ allele *cis* to the $bglR^-$ allele. The merodiploid cells grew on arbutin and salicin medium and showed constitutive P-β-glucosidase B activity with intact cells and cell extracts. However, the constitutive activity of P-β-glucosidase B was five times lower than the activity detected after the cells were induced by β-methyl glucoside.

This indicates that the $bglS4$ allele *cis* to the $bglR^-$ allele forms a soluble product and that it acts in *trans* on the $bglB^+$ gene, but only when the $bglB^+$ allele is expressed *cis* to the regulatory gene ($bglR^-$) function. The results were the same in strain SPF5, where the situation was the same as in strain SPF3 except that the endogenote lacked P-β-glucosidase A activity.

Strain SPF4 possessing the bgl alleles of the following type (F' $bglB^+$, $bglS^+$, $bglR^-$, $bglC^+$/$bglB^+$, $bglS4$, $bglR^+$, $bglC^+$) showed inducible P-β-glucosidase B and transport I activities, which are the function of the genes of the exogenote. It, therefore, appeared that the $bglS4$ allele of the endogenote, due to lack of regulatory gene function ($bglR^+$), failed to determine the formation of soluble product that would have acted in *trans* to allow the $bglC^+$ and $bglB^+$ alleles of the exogenote to be expressed constitutively. In strain SPF6 the situation was the same as in strain SPF4, except that the exogenote lacked the regulatory gene function ($bglR^+$), and thereby the inducible P-β-glucosidase B and transport I activities were not expressed; thus the cells failed to grow on arbutin and salicin medium. This confirmed that in strain SPF4 inducible biosynthesis of P-β-glucosidase B and transport I was due to the expressed $bglB^+$ and $bglC^+$ gene of the exogenote.

Strain SPF15 (F' $bglB^+$, $bglS^+$, $bglR^+$, $bglC^+$/$bglB^+$, $bglS^+$, $bglR^-$, $bglC^-$) failed to grow on

TABLE 7. Merodiploid strains showing P-β-glucosidase B and transport I activities

Strain no.	Merodiploid strain with bgl alleles (exogenote/endogenote)	Activities of P-β-glucosidase[a]		Activities of transport I[b]		Fermentation	
		Constitutive	Induced	Constitutive	Induced	Arbutin	Salicin
SPF2	bgl(A+,B+,S+,R-,C+)/bgl(A+,B+,S+,R+,C+)	–	13			+	+
SPF12	bgl(A+,B+,S+,R-,C+)/bgl(A-,B-,S+,R-,C+)	–	13			+	+
SPF11	bgl(A+,B+,S+,R+,C+)/bgl(A+,B-,S+,R-,C+)	–	–			+	–
SPF10	bgl(A+,B+,S+,R+,C+)/bgl(A+,B-,Sr,R-,C+)	–	–			+	–
SPF3	bgl(A+,B+,S+,R-,C+)/bgl(A+,B-,S4,R-,C+)	2	10			+	+
SPF5	bgl(A+,B+,S+,R-,C+)/bgl(A-,B-,S4,R-,C+)	2	12			+	+
SPF6	bgl(A+,B+,S+,R+,C+)/bgl(A+,B+,S4,R+,C+)	–	–			–	–
SPF4	bgl(A+,B+,S+,R-,C+)/bgl(A+,B+,S4,R-,C+)	–	13			+	+
SPF15	bgl(A+,B+,S+,R+,C+)/bgl(A+,B+,S+,R-,C-)			–	–	–	–
SPF13	bgl(A+,B+,S+,R+,C+)/bgl(A+,B+,S4,ini,R+,C+)			–	–	–	–
SPF14	bgl(A+,B+,S+,R-,C+)/bgl(A+,B+,S4,ini,R+,C+)			4	10	+	+

[a] The enzyme assay was done with the intact cells following the procedure described in Materials and Methods. β-Methyl glucoside (5×10^{-3} M) was used as inducer. Activity is expressed in nanomoles per minute per milligram (dry weight) of cells. The detection with intact cells of enzymatic activity requires the expression of the bglB and bglC genes.

[b] Transport I activity is the same as P-β-glucosidase B activity measured with intact cells. (–), No activity.

arbutin or salicin medium, although the cell extracts possessed the inducible P-β-glucosidase B activity after previous induction with β-methyl glucoside. This indicated that the bglR⁻ allele of the endogenote failed to act in trans to allow the bglC⁺ gene of the exogenote to determine the transport I activity. Strain SPF13 (F' bglB⁺, bglS⁺, bglR⁺, bglC⁺/bglB⁺, bglS4, ini, bglR⁺, bglC⁺) showed only the constitutive P-β-glucosidase B activity with cell extracts due to the presence of the ini mutation, but not with intact cells, and failed to show transport I activity. This indicated that the bglC⁺ allele was not expressed, due to the lack of bglR function on both the exogenote and the endogenote. It thus appeared that the ini mutation allowed the expression of only the bglS4 and bglB⁺ alleles, but not the bglC⁺ allele, of the exogenote and the endogenote. In strain SPF14 (F' bglB⁺, bglS⁺, bglR⁻, bglC⁺/ bglB⁺, bglS4, ini, bglR⁺, bglC⁺) the exogenote possesses the bglB⁺ and bglC⁺ alleles cis to the bglR⁻ allele which is capable of determining the inducible biosynthesis of P-β-glucosidase B and

transport I, whereas the endogenote possesses the bglB⁺ and bglS4 alleles cis to the ini mutation and, therefore, is capable of determining the constitutive biosynthesis of P-β-glucosidase B, even in the absence of bglR function. The merodiploid strain SPF14 showed constitutive transport I activity, indicating the fact that probably the expressed bglS4 allele cis to the ini mutation of endogenote formed a soluble product that acted in trans over expressed bglC⁺ allele cis to the bglR⁻ allele of the exogenote and this determined the constitutive biosynthesis of transport I activity. The bglC⁺ allele of the endogenote was not expressed owing to the lack of regulatory allele function (bglR⁺) even in the presence of the ini mutation.

DISCUSSION

In wild-type strains of E. coli K-12, P-β-glucosidase B (bglB) and β-glucoside transport I (bglC) activities are not detected, owing to the inactivity of their common regulatory gene

function controlled by the *bglR*⁺ locus (12, 14). A mutation at the regulatory site (*bglR*⁺ to *bglR*⁻) causes simultaneous expression of inducible P-β-glucosidase B and transport I activities. In the same strain (*bglR*⁻), an additional mutation (*bglS*⁺ to *bglS4*) allows these enzymes to be formed constitutively (12, 13). Among the strains possessing inducible P-β-glucosidase B and transport I activities [*bgl*(*B*⁺,*S*⁺,*R*⁻,*C*⁺)], two types of transport I-defective mutants were isolated (Fig. 2 [11]).

Mutants of type I failed to express P-β-glucosidase B activity with intact cells or cell extracts and were unable to form Arb⁺ Sal⁺ recombinants with the wild-type strains which lack regulatory gene function (*bglR*⁺). It indicated that the mutants of type I resulted from a back mutation in the regulatory gene *bglR*⁻ leading to *bglR*⁺, which prevented the expression of both P-β-glucosidase B and transport I activities [*bgl*(*B*⁺,*S*⁺,*R*⁺,*C*⁺)]. The lack of recombination with the wild-type strain suggested that the back mutation was at the same site or very close to the wild-type *bglR*⁺ allele. The mutants of type II showed P-β-glucosidase A and B activities with cell extracts, but not with intact cells, and formed Arb⁺ Sal⁺ recombinants with the wild-type strain and with the mutant strain of type I. This suggested that the mutants of type II retained the regulatory gene function (*bglR*⁻) that allowed the expression of the *bglB*⁺ gene to determine P-β-glucosidase B activity, but failed to express transport I activity probably due to a mutation at the structural gene for transport I [*bgl*(*B*⁺,*S*⁺,*R*⁺,*C*⁻)]. This is consistent with the assumption that the wild-type strain possesses unexpressed *bglC*⁺ allele. Mutants of type I were also obtained from strains possessing constitutive P-β-glucosidase B and transport I activities. Similar to the *bglR*⁺ mutants obtained from an inducible strain, the *bglR*⁺ mutants derived from the constitutive strain also lacked P-β-glucosidase B and transport I activities. These results and the results obtained with the merodiploid strains SPF3, SPF5, and SPF4 (Table 7) appear to indicate that the expression of the *bglS4* allele was also under direct control of the *bglR*⁻ allele.

A mutant strain, SP31, was isolated which lacked regulatory gene function due to a back mutation *bglR*⁻ to *bglR*⁺ and, hence, failed to express transport I activity. This strain, despite the lack of *bglR* function, showed a reduced level of constitutive P-β-glucosidase B activity in cell extracts, but intact cells had no detectable activity. The expression of the *bglB*⁺ gene in

the absence of the regulatory effect of the *bglR*⁺ allele was attributed to a mutation designated as *ini*. In strain SP31, the *bglS4* allele was present and expressed as shown by the result obtained with merodiploid strain SPF14 (Table 7). The *ini* mutation was mapped in the vicinity of the *bglS4* allele. Since the *ini* mutation allowed constitutive expression of P-β-glucosidase B activity, which in the initial strain was determined by the expressed *bglS4* allele, it was tentatively assumed that the *ini* mutation might be located to the right of the *bglS4* allele. It appears that the *ini* mutation results in an initiator site that allows the *bglB*⁺ and *bglS4* alleles to determine the expression of constitutive P-β-glucosidase B activities without showing any effect on the expression of the *bglC*⁺ allele. Since the enzyme activity detected in this strain was ten times lower than the parental strain possessing the *bglR*⁻ allele, it is suggested that the *ini* mutation creates a low-level promotor type of function. A similar situation was reported in the *trp* operon of *Salmonella typhimurium*, where the *trpO* and *trpP* genes were deleted by *supX38* mutation and the functioning of the *trpA* and *trpB* genes was renewed by RMX mutation (9). A previous report (13) shows that the *bglR* allele lies between *ilvD* and *bglS*. The present mapping data, resulting from transductional analysis involving three-point cross, show that the *bglC* gene lies between *bglR* and *ilvD* alleles. Thus, the *bgl* genes appear in the following order: *bglB*, *bglS*, *bglR*, *bglC*, and then *ilvD*. For more precise mapping of the *bglC* gene several *bglC* and *bglR* alleles isolated from separate mutations were used.

In Fig. 3, a model is suggested to explain the results obtained from the different mutant strains and from the complementation tests. The regulatory allele (*bglR*⁻), *cis* to the *bglB*⁺, *bglS*⁺ or *bglS4* and *bglC*⁺ alleles, determines simultaneously the expression of inducible or constitutive P-β-glucosidase B and transport I activities. Since the *bglR*⁻ locus lies to the left of the *bglC*⁺ locus and to the right of the *bglS*
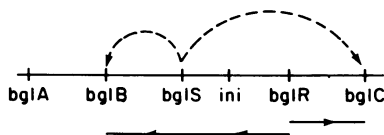

FIG. 3. *A model to explain the effect of regulatory genes (bglR, bglS) on the expression of the bglB and bglC genes to determine the biosynthesis of P-β-glucosidase B and β-glucoside transport I.*

locus, whereas $bglB^+$ lies to the right of $bglS^+$ locus (13), this map order suggests that the mutation $bglR^+$ to $bglR^-$ apparently allows the expression of a regulatory site and allows simultaneously initiation of transcription in opposite directions, to the left to express the $bglC^+$ gene and to the right to express the $bglS4$ and $bglB^+$ genes. The mode of bidirectional transcription has been studied in bacteriophage lambda (17) and at the biotin locus of *E. coli* (5). Bidirectional transcription at the $bglR$ site is explained by two alternative hypotheses: (i) the $bglR^+$ site is composed of two promotors located at opposite strands (18), and upon mutation ($bglR^-$) both promotors are activated simultaneously; and (ii) $bglR^-$ is a single promotor site with broad specificity for the polymerase attachment, composed of two initiators at the opposite sides. Polymerases attach at the promotor and simultaneously initiate transcription at both ends (left and right). Neither of these hypotheses was experimentally tested.

The alleles $bglS^+$ or $bglS4$, $bglB^+$, and $bglC^+$ are expressed only *cis* to the $bglR^-$ allele. The expressed $bglS4$ allele *cis* to the $bglR^-$ allele is dominant over the expressed $bglS^+$ allele and acts in *trans* over the expressed $bglB^+$, $bglC^+$ alleles, determining the constitutive P-β-glucosidase B and transport I activities. In the inducible strain (Table 7) the $bglR^-$ allele *cis* to the $bglS^+$, $bglB^+$, and $bglC^+$ alleles determines inducible P-β-glucosidase B and transport I activities. It therefore appears that the inducer acts at the $bglS^+$ gene which determines the biosynthesis of a regulatory product and which acts in positive control on the structural genes $bglB$ and $bglC$. In the instance of the $bglS4$ allele the cytoplasmic effector is synthesized constitutively, and thus no inducer is required. The strain possessing the $bglS^+$ gene, upon induction, shows a level of P-β-glucosidase B activity approximately two times higher than the strain possessing the $bglS4$ allele. However, when the strain possessing the $bglS4$ allele was induced, the level of P-β-glucosidase B activity was similar to the inducible strain (Table 4). It thus shows that the $bglS4$ allele was incapable of forming cytoplasmic effector to the optimal level. If the cytoplasmic effector was essential to express the $bglB^+$ and the $bglC^+$ genes, the strain possessing the $bglS^-$ allele (no cytoplasmic effector) *cis* to the $bglR^-$ allele should be incapable of forming P-β-glucosidase B and transport I. Since it was technically not possible to isolate a $bglS^-$ mutant, we could not test this hypothesis. However, the experimental results presented in this paper clearly show that the

constitutive biosynthesis of P-β-glucosidase B and transport I is under positive control.

Since Jacob and Monod originally proposed the term operon, it has loosely been defined as a group of contiguous structural genes showing coordinate expression and their closely associated controlling sites (3). Based on this definition, the regulatory genes $bglR$ and $bglS$ and the structural genes $bglC$ and $bglB$, which coordinately express the biosynthesis of P-β-glucosidase B and transport I and lie very close to each other, form a bgl operon.

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