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Biochemistry

β-Glucoside permease represses the *bgl* operon of *Escherichia coli* by phosphorylation of the antiterminator protein and also interacts with glucose-specific enzyme III, the key element in catabolite control

(signal transduction/transport/gene regulation)

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ABSTRACT **The β-glucoside (*bgl*) operon of *Escherichia coli* is subject to both positive control by transcriptional termination/antitermination and negative control by the β-glucoside-specific transport protein, an integral membrane protein known as enzyme II^{Bgl}. Previous results led us to speculate that enzyme II^{Bgl} exerts its negative control by phosphorylating and thereby inactivating the antiterminator protein, BglG. Specifically, our model postulated that the transport protein enzyme II^{Bgl} exhibits protein-phosphotransferase activity in the absence of β-glucosides. We now present biochemical evidence that the phosphorylation of protein BglG does indeed occur *in vivo* and that it is accompanied by the loss of antitermination activity. BglG persists in the phosphorylated state in the absence of β-glucosides but is rapidly dephosphorylated when β-glucosides become available for transport. Our data also suggested specific interactions between the β-glucoside transport protein and the glucose-specific enzyme III (enzyme III^{Glc}), a component of glucose transport and a key element in regulation of catabolite repression. These observations indicate that enzyme III^{Glc} may, in conjunction with enzyme II^{Bgl}, modulate the transport of β-glucosides and the phosphorylation of the antiterminator protein. In the absence of both sugars, when the catabolite-controlled promoter of the operon is derepressed, enzyme III^{Glc} may mediate tight repression of antitermination.**

Wild-type strains of *Escherichia coli* K-12 cannot grow on β-glucosides. The capacity to utilize certain β-glucosidic sugars is acquired by spontaneous mutations. These mutations reveal the presence of an operon, called *bgl*, which is cryptic in the wild-type state. The *bgl* operon encodes all functions necessary for the regulated uptake and utilization of aryl β-glucosides (1). We have determined (2) the nucleotide sequence of the operon and made a functional assignment of its genes (see Fig. 5). The first gene of the operon (*bglG*) encodes a positively acting regulatory (antiterminator) protein. The second gene (*bglF*) encodes the β-glucoside-specific transport protein, enzyme II^{Bgl} (II^{Bgl}) of the phospho*enol*pyruvate:sugar phosphotransferase system (PTS), which phosphorylates β-glucosidic sugars in the process of transporting them through the membrane (Fig. 1; for reviews on the PTS, see refs. 3–5). The third gene *bglB* codes for the catabolic function, a phospho-β-glucosidase. The vast majority of the mutations, which lead to the activation of the operon, turned out to consist of insertions of the insertion elements IS*1* or IS*5* into a proximal region of the operon (2, 6). We have shown that, in the IS*5*-induced Bgl^+ mutants, IS*5* acts as a mobile enhancer, amplifying the activity of the proximal promoter *P0* (see Fig. 5) about 60-fold (7). Inactivity of the operon in the wild-type state thus is due to the weakness of this promoter. Studies in our laboratory and elsewhere have shown that the regulation of the *bgl* operon is quite unusual (7–9). Substrate-dependent induction is not accompanied by modulation of promoter activity but involves alleviation of transcriptional termination at two terminators bracketing gene *bglG*. This operon-specific antitermination is mediated by the protein encoded by *bglG*. Unexpectedly, the β-glucoside transport protein II^{Bgl}, an integral membrane protein, turned out to be a negatively acting regulator. It was clear that, in the absence of substrate, II^{Bgl} exerts its negative effect by somehow interacting with the antiterminator protein, BglG, curtailing its activity. We postulated that, in the absence of substrate, II^{Bgl} catalyzes phosphorylation of the antiterminator protein rendering it inactive (7). We now present evidence in support of this model. We show that protein BglG is indeed phosphorylated *in vivo* in the presence of II^{Bgl} and only in the absence of β-glucosidic substrates. Furthermore, we present data indicating that the glucose-specific enzyme III (III^{Glc}), the cytoplasmic PTS-component of glucose-specific transport (see Fig. 1), modulates both the regulatory and transport activity of II^{Bgl}. III^{Glc} is known to play a central role as a sensor of the catabolic state of the cell and to regulate the activity of adenylate cyclase (10). This regulatory pathway is known as catabolite repression. Our data suggest that III^{Glc}-mediated phosphorylation may contribute to the transport of β-glucosides by II^{Bgl} in the absence of glucose as well as to the inactivation of the antiterminator protein in the absence of both glucose and β-glucosides.

MATERIALS AND METHODS

Bacterial Strains. The following *E. coli* K-12 strains were used: JF201 is Δ*lacX74* Δ(*pho-bgl*) *ara* $B1^-$ (11), R1303 is *galK pro* Δ(*pho-bgl*) with a Tn*10* insertion cotransducing with the *bgl* locus, R1350 is JF201 Δ*crr*::kan^R and R1342 is R1303 Δ*crr*::kan^R. The latter two strains were constructed by transduction of the kanamycin-resistance (kan^R) marker of TP2862 [F^- *xyl argHI* Δ*lacX74 aroB ilvA* Δ*crr*::kan^R (12)].

DNA Manipulations. Plasmids were constructed using standard recombinant techniques (7, 13). The relevant structures of the plasmids used are given in Figs. 2 and 3. The construction of the various plasmids will be described elsewhere and details of the site-specific mutagenesis are given in ref. 14.

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Abbreviations: IPTG, isopropyl β-D-thiogalactopyranoside; MBG, methyl β-D-glucoside; PTS, phospho*enol*pyruvate:sugar phosphotransferase system; II and III, enzyme II and enzyme III (superscripts indicate sugar specificity of enzymes II and III); Bgl, β-glucoside; HPr, heat-stable protein.

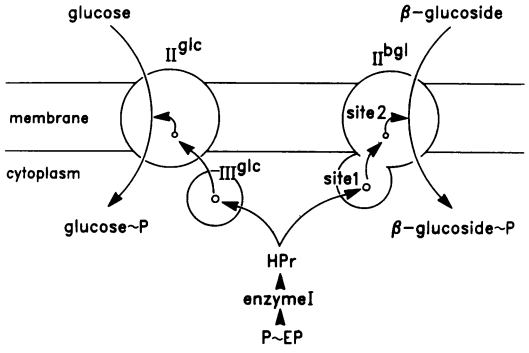

FIG. 1. Two-step phosphorylation pathways of the glucose and β-glucoside transport systems. HPr, heat-stable protein; P~EP, phospho*enol*pyruvate.

Determination of Galactokinase Activity. For galactokinase assays, cells were grown in synthetic (M9) medium (15) containing 1% glycerol, proline (20 μg/ml), Casamino acids (0.66%), ampicillin (50 μg/ml), and tetracycline (12 μg/ml). Where appropriate, isopropyl β-D-thiogalactopyranoside (IPTG; 1 mM) and/or methyl β-D-glucoside (MBG; 5 mM) were added. Aliquots of the cultures were assayed as described (16).

***In Vivo* Protein Labeling.** For labeling with $^{32}PO_4$, cells were grown overnight in LB medium (15) containing ampicillin (50 μg/ml) and diluted to an OD_{600} of 0.2 (≈20-fold) in Tris medium [120 mM Tris·HCl, pH 7.5/80 mM NaCl/20 mM KCl/20 mM NH_4Cl/3 mM Na_2SO_4/1 mM $MgCl_2$/0.2 mM $CaCl_2$/2 nM $FeCl_3$ (17)] containing 1% succinate, 1% glycerol, proline (20 μg/ml), and ampicillin (50 μg/ml). After 3 hr of growth at 37°C, cells were collected by centrifugation and resuspended in the same medium to an OD_{600} of 0.5. Subsequently, 50 μl of this suspension was preincubated for 5 min at 37°C in the presence of 1 mM IPTG, 5 mM MBG, or both, where appropriate. The cells were then labeled with 100 μCi of $[^{32}P]O_4$ (1 Ci = 37 GBq) for 30 min at 37°C and either harvested directly or after a chase with nonlabeled phosphate [by adding (final concentration) 40 mM Na_2HPO_4/20 mM KH_2PO_4/2 mM NH_4Cl] with 5 mM MBG where appropriate. The cells were then incubated in 50 μl of sample buffer (18) for 2 min at 60°C and the labeled proteins were separated by SDS/PAGE on 12.5% polyacrylamide gels (18). After electrophoresis, the gel was subjected to four 15-min washes in a solution of 40 mM Tris phosphate (pH 7.2) containing RNase A (20 μg/ml) to remove the background of labeled RNA, washed several times with the same buffer, dried, and autoradiographed.

RESULTS

Phosphorylation Status of II^{Bgl} Governs Its Activity as a Negative Regulator: Interactions with III^{Glc}. II^{Bgl} belongs to a family of sugar-specific transport proteins of the PTS (19), which share significant homology at the level of amino acid sequence (20, 21). Comparative analysis of these proteins led to the following two conclusions. (*i*) The sugar-specific transport components are either encoded by two genes giving rise to an enzyme II/enzyme III pair [e.g., glucose permease (Fig. 1)] or by one gene whose product is a single polypeptide chain, in which both the enzyme II and the enzyme III domain are combined. The latter is the case for II^{Bgl} (see Fig. 1), which is composed of an N-terminal integral membrane domain (corresponding to II^{Glc}) and a C-terminal enzyme III-like domain. In fact, the C-terminal domain of II^{Bgl} aligns colinearly with III^{Glc} with 34% identity. (*ii*) The homologies between the different transport proteins suggested the presence of two phosphorylation sites in II^{Bgl}: site 1 (histidine-547 of II^{Bgl}) that accepts phosphate groups from the common phosphocarrier pathway of the PTS (phospho*enol*pyruvate → enzyme I → HPr) and site 2 (possibly histidine-306 of II^{Bgl}), which receives the phosphate groups from histidine-547 and transfers them to the sugar molecules as they are transported (Fig. 1; refs. 20 and 21). We have constructed a number of site-specific mutations within gene *bglF* that led to defined amino acid exchanges. The mutations included a histidine to lysine exchange at position 306 (allele *H306K*) and a histidine to arginine exchange at position 547 (allele *H547R*). Analysis of fermentation phenotypes plus *in vitro* assays of transport and sugar phosphorylation indicated that histidine-547 is the postulated site 1 and that histidine-306 is either site 2 itself or another site indispensable for phosphate group translocation (14). In the course of these studies, we found that the defect of allele *H547R* in the transport and concomitant phosphorylation of sugar is complemented by a wild-type *crr* gene, the genetic determinant of III^{Glc}. This indicates that heterologous phosphate group translocation from III^{Glc} to II^{Bgl} takes place (14).

To learn more about II^{Bgl}-mediated negative regulation of the *bgl* operon, we monitored the activity of the antiterminator protein in the presence of wild-type and mutant alleles of *bglF*. In addition to the alleles *H306K* and *H547R*, *bglF-ΔIII*, a deletion of the 3′ end of *bglF* (i.e., the region encoding the enzyme III domain of II^{Bgl}) was tested. The antitermination test system (Fig. 2) consisted of plasmid pFDX1107 [with *bgl* terminator *t2* (7) inserted between constitutive promoter *P16* (22) and the *galK* reporter gene] and a second plasmid carrying gene *bglG* and wild-type or mutant alleles of *bglF* under the control of the *tac* operator promoter (*tacOP*). Double transformants were grown in synthetic medium either with or without IPTG (inducer of the *tacOP*) and either with or without MBG (*bgl*-specific inducer). Aliquots of these cultures were assayed for galactokinase activity. Two strains were used in this experiment: Strain JF201 carries a deletion

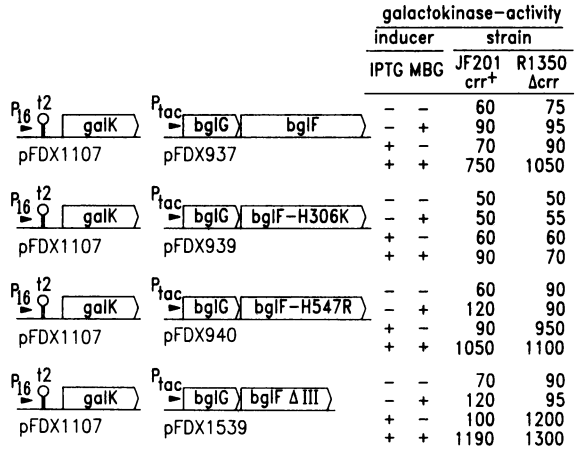

FIG. 2. Inactivation of the antiterminator protein BglG by II^{Bgl}. Relevant plasmid structures are given at the left. Test system consists of an antitermination test plasmid and one of a set of expression plasmids: The antitermination test plasmid (pFDX1107), carrying the constitutive promoter *P16* and a *galK* reporter gene separated by *bgl* terminator *t2*, the *bla* gene, and the *laci*^{q} gene overproducing *lac* repressor, is a derivative of pBR322. The expression plasmids (pFDX937, pFDX939, pFDX940, and pFDX1539) are derivatives of plasmid pACYC177 with the *tet* gene of plasmid pBR322. Expression of *bglG* and alleles of *bglF* in these plasmids is under the control of the *tac* operator-promoter (P_{tac}). The galactokinase activity in units (nmol of galactose phosphorylated per min per OD_{546} unit of bacterial culture) synthesized in the various double transformants ± IPTG and ± MBG (as indicated) is given in the table at the right. Strains JF201 (Δ*bgl*) and R1350 (Δ*bgl* Δ*crr*) were used in this experiment.

of the entire *bgl* operon. Strain R1350 is isogenic with JF201 except for a deletion of *crr*, the gene for III^{Glc}. Fig. 2 gives the relevant plasmid structures accompanied by the experimental results. Plasmid system pFDX1107/pFDX937 directed synthesis of low levels of galactokinase activity in both strains both in the absence of inducers and with either IPTG or MBG alone. In the presence of both inducers, however, about 10-fold more galactokinase was synthesized. These data are in accord with previous findings (7) and demonstrate relief of transcriptional termination by protein BglG in response to MBG. Plasmid system pFDX1107/pFDX939 (carrying allele *bglF-H306K*) gave low levels of galactokinase activity in both strains under all experimental conditions. This shows that a II^{Bgl} defective in phosphorylation site 2 permanently inactivates the antiterminator protein BglG. The system pFDX1107/pFDX940 (carrying allele *bglF-H547R*) gave high levels of galactokinase activity either with IPTG alone or with IPTG in combination with MBG when assayed in the *crr* mutant R1350. This indicates that a II^{Bgl} devoid of phosphorylation site 1 has lost its capacity to inactivate the antiterminator protein BglG in this strain. In the parallel experiment in strain JF201 carrying the wild-type *crr* gene, allele *bglF-H547R* behaved essentially like the wild-type *bglF* allele. Thus a II^{Bgl} devoid of phosphorylation site 1 is efficiently complemented by III^{Glc} in its capacity to negatively regulate activity of the antiterminator protein. Essentially the same results were obtained with plasmid pFDX1539 [carrying allele *bglF-ΔIII*, a deletion of the entire III domain of II^{Bgl} (Fig. 2)].

Phosphorylation of the Antiterminator Protein *in Vivo*. The above results suggest that inactivation of BglG is coupled to the phosphorylation status of site 1 (histidine-547) in II^{Bgl} and demonstrate interaction of II^{Bgl} with III^{Glc}. To test our hypothesis (7) that II^{Bgl} mediates negative regulation of antitermination by phosphorylation of the antiterminator protein (BglG) in the absence of sugar substrate, we monitored BglG phosphorylation *in vivo*. Strains R1303 (deleted for *bgl*) and R1342 (deleted for *bgl* and *crr*) were transformed with plasmids carrying *bglG* and *bglF* or derivatives thereof (Fig. 3). Transformants were incubated in medium containing $H_3[^{32}P]O_4$ and the labeled proteins were separated on an SDS/polyacrylamide gel. The resulting autoradiogram is shown in Fig. 4. In strain R1303, plasmid pFDY42 (carrying genes *bglG* and *bglF* under the control of the *tac* promoter) directed the synthesis of a 29-kDa phosphorylated protein when induced with IPTG (compare lanes 1 and 2). A labeled protein of the same electrophoretic mobility was observed when the experiment was carried out with [^{35}S]methionine (data not shown). Plasmid pFDY49 (carrying a fusion gene

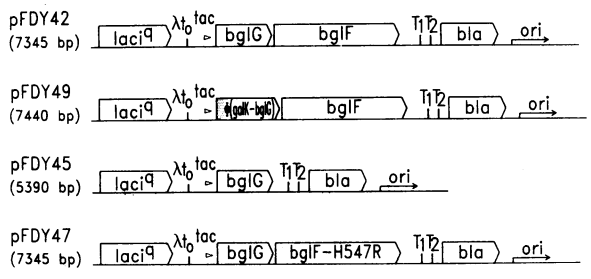

FIG. 3. Relevant structures of plasmids used in *in vivo* phosphorylation studies. The basic plasmid is a derivative of pKK223-3 (23) carrying *laci*^{q}, the *tac* operator-promoter (tac), phage λ terminator *t0*, the *E. coli rrnB* terminators *T1* and *T2*, and the *bla* gene. The various constructions contained the following genes under the control of the *tac* promoter: *bglG* (pFDY45), *bglG-F* (pFDY42), *bglG* and mutant allele *H547R* of *bglF* (pFDY47), the *galK-bglG* fusion gene and *bglF* (pFDY49). The *bglG* gene and the *galK-bglG* fusion gene have the translation start sequence of phage T7 gene *10* derived from plasmid pT7-7 (24). bp, Base pair.

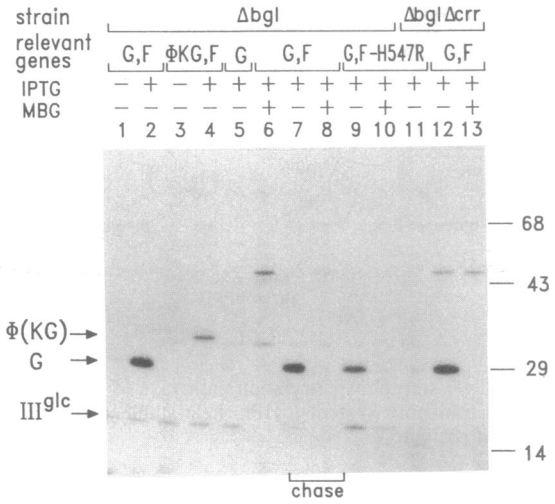

FIG. 4. Phosphorylation of BglG by II^{Bgl} *in vivo*. Transformants were grown with (+) and without (−) IPTG and MBG as inducers for plasmid gene expression and *bgl*-specific antitermination, respectively. Aliquots of the cultures were labeled with radioactive phosphate, separated on SDS/polyacrylamide gels, and autoradiographed. Transformants of strains R1303 (Δbgl) or R1342 (Δbgl Δcrr) were used for labeling. Genes *bglG* and *bglF* and derivatives thereof were expressed under the control of the *tacOP*. Lanes: 1, 2, 6–8, 12, and 13, *bglG* and *bglF* (G, F; plasmid pFDY42); 3 and 4, *galK-bglG* fusion and *bglF* (ΦKG, F; plasmid pFDY49); 5, *bglG* (G; plasmid pFDY45); 9–11, *bglG* and mutant allele *H547R* of *bglF* (G, F-H547R; plasmid pFDY47). Size standards (molecular masses in kDa) are indicated at the right. The positions of proteins III^{Glc} (III^{Glc}), BglG (G), and the GalK–BglG fusion protein [Φ(KG)] are indicated. Lanes 7 and 8 show the results of a pulse–chase experiment. Lanes: 7, 10-min chase with unlabeled phosphate; 8, 1-min chase with unlabeled phosphate in the presence of MBG.

consisting of the full-length *bglG* connected to the 5′ end of *galK* at codon 32) directed the synthesis of a phosphorylated protein with the predicted ≈3.5-kDa shift in molecular mass when IPTG was present (compare lanes 1 and 2 with lanes 3 and 4). A phosphorylated protein of 29 kDa was not detectable in extracts from transformants expressing gene *bglG* alone (plasmid pFDY45; lane 5) or *bglG* and *bglF* (plasmid pFDY42) in the presence of MBG (lane 6). We conclude that protein BglG is indeed phosphorylated *in vivo* in a reaction requiring II^{Bgl} and the absence of β-glucosidic substrates (see Fig. 5). Pulse–chase experiments were performed with transformants of plasmid pFDY42 (carrying *bglG* and *bglF*). Although the signal corresponding to the phosphorylated BglG protein was not significantly diminished after a 10-min chase with unlabeled phosphate (compare lane 7 with lane 2), it completely disappeared after 1 min with unlabeled phosphate and MBG (lane 8). We, therefore, conclude that the phosphorylated BglG protein is not significantly degraded or dephosphorylated in the absence of β-glucosidic substrates of II^{Bgl} but is rapidly dephosphorylated upon the addition of β-glucoside. [Rapid degradation was excluded by pulse–chase experiments performed with [^{35}S]methionine-labeled BglG protein (data not shown).] The results presented in the preceding section (Fig. 2) revealed that the wild-type *crr* gene (encoding III^{Glc}) complements allele *bglF-H547R* in its capacity to inactivate the antiterminator protein BglG. To investigate the correlation of this complementation with the protein phosphotransferase activity of II^{Bgl}, we monitored the phosphorylation of protein BglG by the mutant II^{Bgl} encoded by allele *bglF-H547* (plasmid pFDY47) in a wild-type strain (strain R1303; lanes 9 and 10) and in a strain deficient for III^{Glc} (strain R1342; lane 11). Clearly BglG was only phosphorylated in the presence of III^{Glc} and the absence of MBG (compare lane 9 with lanes 10 and 11). BglG was,

however, phosphorylated in the strain deficient for III^{Glc} (strain R1342) when the mutant *bglF* allele was replaced by the wild-type gene (plasmid pFDY42; lane 12). Again, phosphorylation was curtailed in the presence of MBG (lane 13). Also visible in the autoradiogram is a phosphorylated protein of ≈20 kDa that was only detectable in lanes 1–5 and 9. This band was absent under all conditions in the strain deleted for *crr* (lanes 11–13). Moreover, it was also absent when MBG was available for transport (lanes 6, 8, and 10) and after chasing with unlabeled phosphate (lanes 7 and 8). We conclude that this protein is the phosphorylated form of III^{Glc}, which has a molecular mass of 18.2 kDa in the unphosphorylated state (25), and that III^{Glc} is underphosphorylated in the presence of an inducing β-glucoside. A strongly phosphorylated protein with an apparent molecular mass of ≈45 kDa was visible in lanes 6, 12, and 13. This protein was also phosphorylated in strains lacking the PTS system (i.e., deleted for the genes encoding enzyme I and HPr; data not shown). The phosphorylated form of II^{Bgl} was not observed under the conditions of this experiment.

DISCUSSION

We have investigated the mechanism, by which the β-glucoside permease (II^{Bgl})—an integral membrane protein—acts as a negative regulator of the *bgl* operon in the absence of its substrate. Our model for *bgl* regulation (7), as extended by the data presented here, is illustrated in Fig. 5.

The inactivation of the *bgl* antiterminator protein (protein BglG) requires (*i*) the presence of II^{Bgl}, the β-glucoside-specific transport protein, and (*ii*) the absence of the β-glucosidic substrates of the transport protein (7, 9). II^{Bgl} phosphorylates its substrate in the process of transporting it through the membrane (19). Since II^{Bgl} is itself phosphorylated by the common components of the PTS (14, 20, 21), we reasoned that the capacity of II^{Bgl} to inactivate BglG must be linked to the phosphorylation status of II^{Bgl}, which in turn reflects the availability of β-glucosides. We, therefore, tested a mutant of II^{Bgl} with an amino acid exchange at phosphorylation site 1 and a mutant blocked at phosphorylation site 2 (alleles *H547R* and *H306K*, respectively) for their capacity to inactivate BglG. As can be seen from the data given in Fig. 2, II^{Bgl}-H306K, in which phosphorylation of site 2 is blocked resulting in permanent phosphorylation of site 1, rendered BglG inactive under all experimental conditions. To our surprise we found that, in a wild-type strain, the mutant II^{Bgl}-H547R (site 1 negative) behaved essentially like the wild-type II^{Bgl} both in its capacity to negatively regulate antitermination (Fig. 2) and in its capacity to transport β-glucosides (14). A completely different picture emerged, however, with a strain deleted for *crr* and thus deficient in the cytoplasmic component of glucose transport, III^{Glc}. In this strain II^{Bgl}-H547R had no inhibitory effect on BglG (Fig. 2) and there was no transport of β-glucosides (14). A mutant II^{Bgl} with a C-terminal deletion spanning the entire region of homology to III^{Glc} behaved similarly. Thus III^{Glc} efficiently complements a defect of the C-terminal domain of II^{Bgl} in its transport and regulatory functions. Thus these results suggest that inactivation of BglG is coupled to the phosphorylation status of site 1 (histidine-547) according to the following scenario: In the presence of transportable substrate, the phosphate groups donated from the common components of the PTS are translocated from site 1 through site 2 to the sugar molecules as they are transported, resulting in a net underphosphorylation of site 1. When no β-glucosides are present or when site 2 is blocked (allele *H306K*), phosphate groups can no longer be drained from site 1, causing it to be permanently phosphorylated. This led us to speculate that, in the absence of phosphorylatable sugar, II^{Bgl} might phosphorylate and thereby inactivate BglG (7). To test this hypothesis

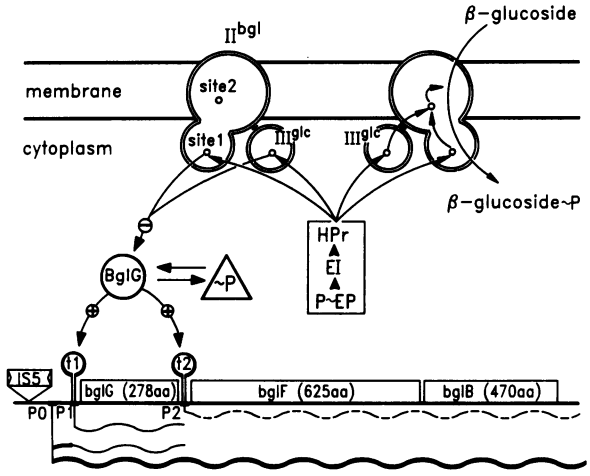

FIG. 5. Model for the regulation of the *bgl* operon. The mutationally activated promoter *P0* is subject to modulation by the catabolite gene repression system but not by β-glucosides as substrates. Induction by β-glucosidic substrates is mediated by modulation of termination/antitermination at terminators *t1* and *t2*, which flank gene *bglG*. Protein BglG mediates antitermination at both sites. BglG-mediated antitermination is negatively controlled by the product of gene *bglF*, the β-glucoside specific transport protein known as II^{Bgl}. II^{Bgl} also interacts with III^{Glc}, the cytoplasmic component of the glucose transport complex. We postulate the following scenarios: (*i*) In the presence of β-glucoside but absence of glucose the phosphate groups are translocated from phosphorylation site 1 (histidine-547 in II^{Bgl}) by way of phosphorylation site 2 to the sugar molecules as they are transported. This drain of phosphate groups leads to a net underphosphorylation of site 1. III^{Glc} donates phosphate groups to phosphorylation site 2 thereby enhancing β-glucoside transport. (*ii*) When glucose becomes available III^{Glc} switches to glucose transport resulting in an immediate slow-down in β-glucoside transport (see Fig. 1). (*iii*) When both β-glucoside and glucose are absent, phosphate groups at site 1 cannot be drained by site 2 to the sugar leaving site 1 permanently phosphorylated. Under these conditions II^{Bgl} assumes protein-phosphotransferase activity and the phosphate groups are transferred from site 1 to BglG leading to its inactivation. III^{Glc} also contributes to the phosphorylation of BglG thereby mediating more efficient inactivation of BglG and hence a more stringent inhibition of antitermination. This tight control is essential because the absence of glucose leads to a stimulation of the catabolite-controlled *bgl* promoter *P0* and maintenance of the repressed state depends on efficient termination. BglG persists in the phosphorylated state in the absence of sugar substrate but is rapidly dephosphorylated when β-glucosides become available. For additional details see ref. 7. EI, enzyme I; P~EP, phospho*enol*pyruvate; aa, amino acid(s).

we constructed an *in vivo* system in which gene *bglF* was expressed with gene *bglG* (or with a hybrid *bglG* gene consisting of the 5′ end of the *galK* gene fused in frame to the 5′ end of the full-length *bglG* gene) in the presence of $[^{32}P]O_4$. Phosphorylation of protein BglG could indeed be demonstrated. Moreover, phosphorylation took place only in presence of an intact *bglF* gene and in the absence of β-glucosides (Fig. 4). These results provide direct biochemical evidence for our model (Fig. 5). Furthermore, pulse–chase experiments indicated that BglG remains in the phosphorylated state as long as the sugar substrates of II^{Bgl} are absent but is rapidly dephosphorylated upon their addition (Fig. 4). This rapid dephosphorylation suggests that reactivation of preformed but inactive BglG contributes to induction of the *bgl* operon. It also suggests that the transfer of phosphate groups from II^{Bgl} to BglG is reversible and can proceed in the opposite direction when II^{Bgl} is engaged in sugar transport.

Also included in the schematic representation of the model in Fig. 5 are the interactions of III^{Glc} with II^{Bgl} deduced from complementation studies with a II^{Bgl} mutant defective in the

enzyme III domain. Recent observations on the complementation of a defective III^{Glc} by an intact II^{Bgl} (14, 27) provide additional evidence for such true cross-talk between the two systems. Furthermore, II^{Bgl} is capable of transporting and phosphorylating glucose even in the absence of a functional $II–III^{Glc}$ pair (14). Although additional data are needed to clarify the biological significance of the interactions between II^{Bgl} and III^{Glc}, we find the following hypothesis attractive: III^{Glc} contributes to and thereby enhances transport of β-glucosides in the absence of glucose. When glucose is added, III^{Glc} plus II^{Glc} are occupied in glucose transport so that III^{Glc} is not available for β-glucoside transport. Thus, an immediate slow-down of β-glucoside transport occurs. Under these conditions (or when β-glucosides are absent) II^{Bgl} may in turn contribute to glucose transport. This would mediate a rapid adaptation of the catabolite network in favor of glucose. Why, then, does III^{Glc} also contribute to the inactivation of BglG? The *bgl* operon promoter *P0* (see Fig. 5) is under the stringent control of catabolite repression (ref. 11; unpublished results). Therefore, promoter *P0* is inactive in the presence of glucose and efficient termination is not required. However, when neither glucose nor a β-glucoside is present, promoter *P0* will be stimulated by the cAMP–catabolite repressor protein complex. Under these conditions maintenance of the repressed state depends on efficient termination and hence on maximal inactivation of the antiterminator BglG. If the above hypothesis is correct, it follows that the *bgl* operon should be inducible by glucose in a wild-type strain when promoter *P0* is replaced by a constitutive catabolite-insensitive promoter. Our experimental evidence (to be published elsewhere) confirms this prediction.

III^{Glc} is known to be a key component of catabolite regulation. Its phosphorylation status governs the activity of adenylate cyclase. Thus III^{Glc} participates indirectly in the regulation of a number of different operons (10). This type of regulation is relatively slow, since the shutting down of secondary catabolic pathways in response to the availability of glucose requires dilution or turn-over of the preformed components of these pathways. The activity of several other non-PTS sugar permeases (e.g., lactose permease) and uptake systems (e.g., glycerokinase, which traps glycerol by phosphorylation) may also be subject to control by the nonphosphorylated form of III^{Glc} (for review, see ref. 5). II^{Bgl} may represent another case of partial inhibition of a transport protein by III^{Glc} in response to glucose. In addition, III^{Glc} participates in the regulation of the *bgl* operon at the level of transcriptional termination/antitermination. III^{Glc} thus appears to be a multifaceted protein active on different levels of the global control network of *E. coli*.

Note. After this manuscript was submitted, Amster-Choder *et al.* (26) reported reversible *in vitro* phosphorylation of BglG by II^{Bgl}. In general their data are in accord with the results presented here.

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