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Glucose Transporter Mutants of *Escherichia coli* K-12 with Changes in Substrate Recognition of IICB^{Glc} and Induction Behavior of the *ptsG* Gene

TIM ZEPPENFELD, CHRISTINA LARISCH, JOSEPH W. LENGELER, AND KNUT JAHREIS*
Arbeitsgruppe Genetik, Fachbereich Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany

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In *Escherichia coli* K-12, the major glucose transporter with a central role in carbon catabolite repression and in inducer exclusion is the phosphoenolpyruvate-dependent glucose:phosphotransferase system (PTS). Its membrane-bound subunit, IICB^{Glc}, is encoded by the gene *ptsG*; its soluble domain, IIA^{Glc}, is encoded by *crr*, which is a member of the *pts* operon. The system is inducible by D-glucose and, to a lesser degree, by L-sorbose. The regulation of *ptsG* transcription was analyzed by testing the induction of IICB^{Glc} transporter activity and of a single-copy Φ(*ptsGop-lacZ*) fusion. Among mutations found to affect directly *ptsG* expression were those altering the activity of adenylate cyclase (*cyaA*), the repressor DgsA (*dgsA*; also called Mlc), the general PTS proteins enzyme I (*ptsI*) and histidine carrier protein HPr (*ptsH*), and the IIA^{Glc} and IIB^{Glc} domains, as well as several authentic and newly isolated UmgC mutations. The latter, originally thought to map in the repressor gene *umgC* outside the *ptsG* locus, were found to represent *ptsG* alleles. These affected invariably the substrate specificity of the IICB^{Glc} domain, thus allowing efficient transport and phosphorylation of substrates normally transported very poorly or not at all by this PTS. Simultaneously, all of these substrates became inducers for *ptsG*. From the analysis of the mutants, from *cis-trans* dominance tests, and from the identification of the amino acid residues mutated in the UmgC mutants, a new regulatory mechanism involved in *ptsG* induction is postulated. According to this model, the phosphorylation state of IIB^{Glc} modulates IIC^{Glc} which, directly or indirectly, controls the repressor DgsA and hence *ptsG* expression. By the same mechanism, glucose uptake and phosphorylation also control the expression of the *pts* operon and probably of all operons controlled by the repressor DgsA.

In *Escherichia coli* K-12, D-glucose (Glc) is taken up and concomitantly phosphorylated either by the glucose-specific enzyme II (EII) transporter (II^{Glc}) or the mannose-specific EII transporter (II^{Man}) (genes *manXYZ*) of the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) (for reviews, see references 10 and 21). As for most other PTS carbohydrates, the phosphoryl groups are sequentially transferred from PEP through two common intermediates, enzyme I (EI; gene: *ptsI*) and the phosphohistidine carrier protein (HPr; gene: *ptsH*), to sugar-specific EII (IICB^{Glc}; see below) and to glucose (for a review see reference 41).

II^{Glc} consists of two subunits, IIA^{Glc} (*crr* [catabolite repression resistance]) and membrane-bound IICB^{Glc} (*ptsG*) (8). The *crr* gene is part of the *ptsHI crr* operon (46) separated from the *ptsG* gene, which maps at 25.0 min (4). IIA^{Glc} is a small hydrophilic protein which has, in addition to its transport function, a central regulatory role in carbon catabolite repression and inducer exclusion (for a review, see reference 22). The IICB^{Glc} subunit is composed of an amino-terminal, hydrophobic IIC^{Glc} domain, which largely determines substrate specificity, and a carboxy-terminal, hydrophilic IIB^{Glc} domain, which is phosphorylated at the Cys421 residue (32). The system normally recognizes glucose as well as methyl-α-D-glucoside (αMG), 5-thio-D-glucoside, L-sorbose and, with a low affinity, 2-deoxyglucose (2DG) (for a review, see reference 40).

Only little attention has been paid to the regulation of *ptsG* expression, although IICB^{Glc} has an outstanding regulatory function in establishing glucose as a favored carbon source. Moreover, for both *E. coli* (45) and *Salmonella enterica* serovar Typhimurium (55), it was demonstrated that the activity of IICB^{Glc} is the rate-limiting step in glucose utilization. Both *ptsG* expression (19, 37, 43) and *manXYZ* expression (36) are positively regulated by the cyclic AMP (cAMP)-cAMP receptor protein (CrpA) complex and negatively controlled by the DgsA (Mlc) protein. The *dgsA* locus (deoxyglucose sensitive) at 35.9 min on the *E. coli* chromosome was discovered as a suppressor mutation that enables *ptsG*-negative mutants to grow anaerobically on glucose via a constitutively expressed II^{Man} system and enhanced sensitivity to 2DG, a major substrate of this transport system (44). The DgsA protein was rediscovered recently and renamed Mlc (making large colonies) (16). Plumbridge (36) demonstrated that *dgsA* and *mlc* are the same gene; for priority reasons and according to Berlyn (4), we call this gene *dgsA*. The DgsA protein represses its own synthesis as well as the expression of the *ptsHI crr* operon (18, 38) and the *mal* regulon (7). It may represent a novel global repressor and may counteract the global regulator cAMP-CrpA to ensure the expression of those genes, which are linked to glucose metabolism (19, 38, 39, 44). The inducer for DgsA, however, has not been identified.

A different type of *ptsG* regulatory mutation, called *umgC* (uptake of αMG control; *umg* is the former name of *ptsG*), was described by Jones-Mortimer and Kornberg (17). This mutation, which was claimed to map close to but not in *ptsG*, enabled *E. coli* cells with inactive II^{Man} to grow on mannose (Man) and glucosamine (GlcN or Glm). The authors concluded that mannose and glucosamine are not inducers of the glucose PTS, that the *umgC* mutation causes constitutive *ptsG*

* Corresponding author. Mailing address: Arbeitsgruppe Genetik, Fachbereich Biologie/Chemie, Universität Osnabrück, D-49069 Osnabrück, Germany. Phone: 49-541-969-2288. Fax: 49-541-969-2293. E-mail: Jahreis@Biologie.Uni-Osnabrueck.de.

expression, and that *umgC* encodes a repressor, UmgC. In this paper, we describe the isolation and characterization of UmgC-like mutants which were selected as described by Jones-Mortimer and Kornberg (17). Moreover, we reinvestigated one of their UmgC mutants and showed that *umgC* mutations map within the *ptsG* allele and alter characteristically the *ptsG* induction pattern and $IICB^{Glc}$ transporter activity.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The *E. coli* K-12 strains and plasmids used in this study are listed in Table 1. LJ110 (Fnr^+) was obtained from a cross of K-12(P1) with W3110 and by selection for growth on minimal agar plates supplemented with 0.2% glycerol and 10 mM KNO_3 under anaerobic conditions. Cells were routinely grown either in standard phosphate minimal medium (54) supplemented with 0.2% various carbon sources, in Lennox broth without glucose and calcium ions, or in 2xTY medium as described by Ausubel et al. (2). The utilization of various carbohydrates was screened on MacConkey agar plates (Difco) containing 1% of the indicated carbon source. Antibiotics were used at the following concentrations: tetracycline, 10 mg/liter; ampicillin, 50 mg/liter; chloramphenicol, 25 mg/liter; kanamycin, 25 mg/liter; and spectinomycin, 1,500 mg/liter for the multicopy plasmid system or 100 mg/liter in rich medium and 500 mg/liter in minimal medium for the single-copy system. Transductions were carried out with P1 *vir* essentially as described by Arber (1).

Construction of single-copy Φ(*ptsGop-lacZ*) and Φ(*ptsHp-lacZ*) fusions. For the construction of a single-copy Φ(*ptsGop-lacZ*) fusion, the promoter-operator region was amplified from genomic DNA of LJ110 by PCR using the oligonucleotides ptsG4 (5′-AATCAACCTGCGATGGTTCC-3′; hybridizing to bp −337 to −318 upstream of the *ptsG* start codon) and ptsG3 (5′-AATACCTGCGATAGGCAGTACGGATACCGG-3′, hybridizing to codons 19 to 28 for $IICB^{Glc}$). The product was treated with Klenow DNA polymerase to produce blunt ends and was inserted into the *Eco*RV restriction site of pTIM101. Vector pTIM101 essentially is a derivative of plasmid pIC-19H (26) in which the multiple cloning site was deleted. It carries a truncated Tn*1721* transposon (52) which consists of the inverted repeat regions, the multiple cloning site of pBluescript II SK(+) (*Hae*II box) (2), and the so-called Ω element of plasmid pHP45Ω (42). The Ω element provides transcriptional and translational stop signals to prevent read-through from any potential upstream promoter and an *spc* gene for spectinomycin and streptomycin resistance. The orientation of the PCR insert was controlled by DNA sequencing. Downstream of the *ptsG* promoter region, the promoterless *lacZ* gene from plasmid pRU869 (53) was inserted into the *Hin*dIII/*Sal*I restriction sites to produce plasmid pTIM103.

For the construction of a single-copy Φ(*ptsHp-lacZ*) fusion, the promoter-operator region was amplified from genomic DNA of LJ110 by PCR using the oligonucleotides pts1 (5′-GATCTCTTCACTGAGAAAGAATTGC-3′, hybridizing to codons 313 to 321 of CysK) and pts2 (5′-ACATTGTATTTCCCCAACTTATAGG-3′, hybridizing to 21 bp upstream of *ptsH* and codon 1 of HPr). The 420-bp fragment was treated with Klenow DNA polymerase to produce blunt ends and was inserted into the *Eco*RV restriction site of pTIM101. The orientation of the PCR insert was controlled by DNA sequencing. Downstream of the *ptsH* promoter region, the promoterless *lacZ* gene from plasmid pRU869 was inserted into the *Hin*dIII/*Sal*I restriction sites to produce plasmid pTIM104.

PS8/F′8 (gal^+) (11) was transformed with plasmids pTIM103 and pTIM104. To allow transposition onto the F′8 plasmid, cells were transformed with plasmid pPSO110 (54), which carries the gene for the Tn*1721* transposase. F′ plasmids containing the Φ(*ptsGop-lacZ*) or Φ(*ptsHp-lacZ*) transcriptional fusions on the truncated, artificial transposon were transferred into appropriate test strains. Loss of mobilized pTIM103 or pTIM104 was controlled by simultaneous loss of Ap^r.

Isolation of plasmid DNA, restriction analysis, and cloning procedures. All manipulations with recombinant DNA were carried out using standard procedures as described previously (2). Plasmid DNA was prepared either by using standard phenol extraction protocols as described previously (48) or by using the JETstar DNA purification system (Genomed, Bad Oeynhausen, Germany). Restriction enzymes were purchased from New England Biolabs (Schwalbach, Germany). They were used according to the recommendations of the supplier. Oligonucleotides for sequencing or PCR were purchased from Interactiva (Ulm, Germany).

Mutation analysis. DNA amplification of the *ptsG* alleles was done as described by Saiki et al. (47) using *Taq* DNA polymerase from Roche Diagnostics, Mannheim, Germany, or Goldstar polymerase from Eurogentec, Seraing, Belgium. The forward PCR primer ptsG+ (5′-AACTGCAG**GTG**TTTAAGAATGCATTTGCT-3′) for the amplification of *ptsG* contained an engineered *Pst*I restriction site (underlined) upstream of an artificial GTG start codon (boldface; the original ATG was changed to GTG to lower the levels of expression of *ptsG* after subcloning into pSU19 [see below]). The reverse PCR primer ptsG− (5′-CTTAAAGCTTAGTGGTTACGGATGTA-3′) introduced an engineered *Hin*dIII restriction site (underlined) immediately downstream of the TAA stop codon (Fig. 1). The reaction profile consisted of 32 cycles of denaturing at 94°C for 1 s, annealing at 50°C for 1 s, and extension at 72°C for 45 s in an Air Thermo-Cycler 1605 from Idaho Technology Inc., Idaho Falls, Idaho. PCR products were directly purified using a Wizard PCR Preps DNA purification system (Promega Corp., Mannheim, Germany). All DNA sequencing reactions were performed by the dideoxy chain termination method (2) using an ALFexpress AutoRead or dATP labeling mix sequencing kit from Amersham-Pharmacia Biotech, Freiburg, Germany. The nucleotide sequences of both strands were determined after subcloning into vector pSU19 (27) using 5′ cyanine fluorescent dye (Cy-5)-labeled universal and reverse primers or unlabeled internal *ptsG* sequencing oligonucleotides priming about every 250 bp within the gene. Computer analysis was done with DNASIS sequencing analysis software (Hitachi) and by using the BLAST programs and database services provided by the National Center for Biotechnology Information, Bethesda, Md.

Construction of defined carboxy-terminal deletions in $IICB^{Glc}$. Plasmid pSTJ30Δ320 is a derivative of pTM110 which was digested with restriction enzymes *Stu*I (bp 957 to 963 in *ptsG*) and *Hin*dIII (in the polylinker region downstream of *ptsG*) (Fig. 1). DNA was treated with Klenow DNA polymerase to generate blunt ends and was religated. The open reading frame encoded a protein which consisted of up to amino acid 320 of $IICB^{Glc}$ and six additional, plasmid-encoded amino acids (AWYLTN). All other pSTJ30 deletion plasmids were generated using pTM110 as a template, the ptsG+ primer as an upstream primer, and an appropriate downstream primer in a PCR. The following reverse PCR primers were used for the different deletion plasmids: for pSTJ30Δ396, ptsG21 (5′-CCTGAAGCTTTTGCATCTTCAGTCG-3′); for pSTJ30Δ436, ptsG22 (5′-TGATAAGCTTTAGACACATCAGCAA-3′); and for pSTJ30Δ459, ptsG23 (5′-GAAAAGCTTCTGAACACCAGAACC-3′). All primers created an artificial *Hin*dIII restriction site (underlined). The last number in each plasmid name indicates the last original PtsG amino acid encoded by the particular construct. PCR fragments were treated with *Hin*dIII and *Pst*I and cloned into pTM30. All plasmid constructs were confirmed by DNA sequencing. The addition of at least 50 μM isopropyl-β-D-thiogalactopyranoside (IPTG) to cells carrying these deletion plasmids led to complete growth inhibition, indicating that truncated proteins were expressed from these plasmids and, like wild-type PtsG, were lethal for the cells (data not shown).

Transport and enzyme assays. Transport of α-D-[*methyl*-^{14}C]glucopyranoside (final concentration, 25 μM) was measured in exponentially grown cells as described previously (49). Samples were taken after 10, 20, and 30 s. Transport activities were calculated from the initial uptake rates. The β-galactosidase assay was performed as described by Pardee and Prestige (34). All enzyme activities are in nanomoles per milligram of protein per minute.

RESULTS

Isolation and characterization of UmgC mutants of *E. coli*. Our isogenic *E. coli* derivatives which lack the II^{Man} transporter due to a mutation in the *manA* locus (genes *manXYZ*) (9) are unable to utilize D-glucosamine (GlcN or Glm) or D-mannose (Man) as a sole carbon source (Table 2). Cells of the $ManA^-$ mutants LJ130 and LJ132 had to be incubated for 3 days at 37°C on minimal glucosamine plates before small colonies appeared. One hundred of these were isolated, purified, and tested for their ability to grow on various carbohydrates. The majority (80%) of the isolated colonies had a pleiotropic phenotype, i.e., they became Glm^+ Man^+ and at the same time became sensitive to D-arabinitol (Atl^s) and ribitol (Rtl^s). Each of these four carbohydrates has been discussed to be a minor gratuitous substrate for $IICB^{Glc}$, and transport occurs only when the transporter is expressed constitutively (reviewed in references 39 and 40). No degradation pathways for Atl-5-phosphate and Rtl-5-phosphate are present in *E. coli* K-12, thus explaining the sensitivity (14). Two mutants, LZ1 and LJ132-3, derived from LJ130 and LJ132, respectively, were further characterized, compared to an authentic UmgC mutant (HK727), and used to map the new mutations (Table 2).

Mapping by P1 transduction for both LZ1 and LJ132-3 placed the new mutations close to *zce-726*::Tn*10* (95% coupling to *ptsG*) and also close to or in *ptsG* (data not shown). Strain HK727 carries an authentic UmgC mutation, a $ManA^-$ allele, the *ptsI19*(Ts) allele, and a *crr*(Ts) mutation, which prevent growth on PTS carbohydrates at temperatures above 30°C (30, 35). As expected for a strain lacking II^{Man} but carrying the UmgC mutation, HK727 is able to grow on GlcN plates; like LZ1 and LJ132-3, it is also Man^+ Atl^s Rtl^s. When incubation is done at 42°C, this phenotype changes to Glm^- Man^- Atl^r Rtl^r, indicating that cells are sensitive to the two pentitols only

TABLE 1. *E. coli* K-12 strains and plasmids used in this study[a]

Strain or plasmid	Relevant genotype or phenotype	Reference or source
Strains		
CAG12078	F^- *zce-726*::Tn*10*	50
ZSC112LΔG	F^- Glk^- Δ(*ptsG*::*cat*)	6
ZSC112LΔLPM	F^- Glk^- Δ(*manXYZ*::*cat*)	12
JWL359	F^- Δ*lacU169 zah-735*::Tn*10*	J. W. Lengeler
JM1100	Hfr *man-8*	13
PS8	F^- Δ*lacU169 recA56*	56
TP2811	F^- Δ(*ptsHI crr*::*kan*)	25
W3110	F^- Fnr^-	15
LJ110	W3110 Fnr^+	This study
LJ120	Δ(*ptsG*::*cat*)	This study
LJ121	Δ(*ptsG*::*cat*) *man-8 zea-225*::Tn*10*	This study
LJ130	LJ110 Δ(*manXYZ*::*cat*)	This study
LJ132	LJ130 *zce-726*::Tn*10*	This study
LJ132-3	LJ132 $ptsG_3$	This study
LJ333	LJ132-3 $ptsG_{33}$	This study
LZ1	LJ130 $ptsG_1$	This study
LZ22	LZ1 $ptsG_{22}$	This study
LZ23	LZ1 $ptsG_{23}$	This study
LZ100	LZ1 $ptsG_1$ Δ*lacU169 zah-735*::Tn*10*	This study
LZ110	LJ110 $ptsG^+$ Δ*lacU169 zah-735*::Tn*10*	This study
LZ120	LZ110 Δ*cyaA854*	This study
LZ140	LZ110 Δ(*ptsHI crr*::*kan*)	This study
LZ150	LZ110 Δ(*ptsG*::*cat*)	This study
LZ160	LZ110 Δ(*ptsG*::*cat*) Δ(*ptsHI crr*::*kan*)	This study
HK727	F^- $ptsG_{727}$ *ptsI19*(Ts) *crr*(Ts) $manXYZ_{727}$	35
LZ727	LJ130 $ptsG_{727}$ *zce-726*::Tn*10*	This study
JWL184-1	F^- $ptsG_{184}$ $manXYZ_{184}$	23
JWL184-20	JWL184-1 Δ(*ptsG*::*cat*)	This study
JWL184-30	JWL184-1 Δ(*manXYZ*::*cat*)	This study
KM563	F^- *dgsA*::Tn*10kan*	7
LJ138	LJ130 *dgsA*::Tn*10kan*	This study
LZ138	LJ138 $ptsG^+$ Δ*lacU169 zah-735*::Tn*10*	This study
LZ139	LZ138 Δ(*ptsG*::*cat*)	This study
LZ170	LZ1 *dgsA*::Tn*10kan*	This study
Plasmids		
pSU19	Cm^r	27
pPSO110	$tnpA^+$ Cm^r	54
pJBH	Ap^r	6
pJCH	Ap^r	6
pTM30	Ap^r	29
pTM110	Ap^r $ptsG^+$	This study
pTM1	Ap^r $ptsG_1$	This study
pTM32-8	Ap^r $ptsG_3$	This study
pTM727	Ap^r $ptsG_{727}$	This study
pTM184-1	Ap^r $ptsG_{184}$	This study
pSTJ30Δ320	Ap^r Δ$ptsG_{320}$	This study
pSTJ30Δ328	Ap^r Δ$ptsG_{328}$	This study
pSTJ30Δ396	Ap^r Δ$ptsG_{396}$	This study
pSTJ30Δ436	Ap^r Δ$ptsG_{436}$	This study
pSTJ30Δ459	Ap^r Δ$ptsG_{459}$	This study
pTIM101	Ap^r Sc^r	This study
pTIM103	Ap^r Φ(*ptsGop-lacZ*)	This study
pTIM104	Ap^r Φ(*ptsHop-lacZ*)	This study
F′8 (gal^+)	gal^+	11
F′8::Tn Φ(*ptsGop-lacZ*)	Sc^r	This study
F′8::Tn Φ(*ptsHop-lacZ*)	Sc^r	This study

[a] The nomenclature for the *pts* genes is that of Postma et al. (40); the nomenclature for other genes is that of Berlyn (4). For clarity, *ptsG* or *manXYZ* mutations in the *manA* locus from different strains were named according to their source (subscript numbers). The *dgsA* (2DG sensitivity) gene encodes the repressor DgsA or Mlc (making large colonies).

in the presence of a functional PTS. To differentiate between PTS- and UmgC-dependent effects, the $ptsG_{727}$ allele from HK727 was transduced into LJ130 after the *zce*-727::Tn*10* cassette from CAG12078 was transferred into HK727. The majority (76%) of Tet^r transductants of LJ130 (e.g., LZ727 in Table 2) exhibited a temperature-resistant Pts^+ phenotype and were Glm^+ Man^+ Atl^s Rtl^s, i.e., the UmgC phenotype. According to these mapping data, mutants LZ1 and LJ132-3 thus seem to correspond to authentic UmgC mutants. Any further attempts to uncouple the *ptsG* and *umgC* markers in LJ132-3,

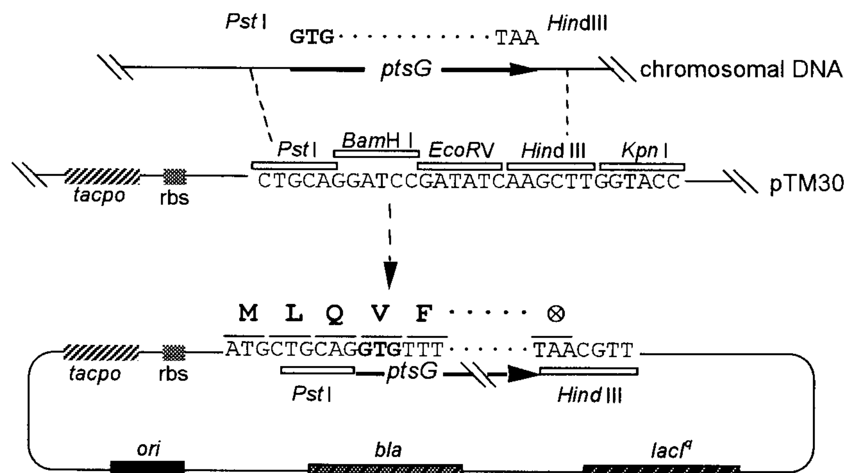

FIG. 1. Construction of *ptsG* expression plasmids. The *ptsG* alleles from various strains were amplified by PCR as described in Materials and Methods, introducing artificial *Pst*I (upstream) and *Hin*dIII (downstream) restriction sites. The original ATG start codon of *ptsG* was changed to GTG (boldface). Fragments were subcloned into pTM30 cut with *Pst*I and *Hin*dIII in frame with an artificial ATG start codon provided by the expression vector. All constructs provided three additional, amino-terminal amino acid residues. The expression vector also provided a strictly regulated *tac* promoter-operator (*tacpo*), a $lacI^q$ gene, and a ribosome binding site (rbs) in an optimal position with respect to the start codon.

LZ1-2, or LZ727 failed (data not shown). Thus, a mutation in *ptsG* or a mutation very close to it seems to be responsible for GlcN, Man, Rtl, and Atl uptake in the UmgC mutants.

Exact growth rates were determined for the various wild-type and mutant strains on different carbon sources (Table 2). Strains included a derivative of LJ130 carrying a defined *dgsA*::Tn*10kan* mutation from KM563. This mutant, LJ138, did not show a UmgC phenotype, although it expressed *ptsG* in a constitutive way (see below). Thus, a change in the substrate specificity of $IICB^{Glc}$ rather than constitutive expression of *ptsG* was responsible for the characteristic change in UmgC mutants. The generation times for LJ110 and isogenic derivatives on glycerol (90 to 96 min) and on glucose (72 to 75 min) were almost equal, except for the $DgsA^-$ mutant, which had a generation time of 115 min on glycerol. This result seems to corroborate the hypothesis that DgsA is a global rather than a glucose-specific regulator (7, 38, 39). While growth on mannose was relatively similar for a $ManA^+$ strain and a UmgC mutant, growth of the latter on glucosamine was retarded (210 and 280 min compared to 126 min). Finally, the addition of 1% ribitol or arabinitol to cells of LZ1 or LJ132-3 growing exponentially on glycerol caused growth inhibition after three cell division cycles, while mutant but not wild-type cells preinduced with glucose stopped growing almost immediately (data not shown). The induction of *ptsG* by the two pentitols in the mutants but not in the wild type was responsible for this effect (see below).

Induction of *ptsG* expression in various isogenic mutants. To test the *ptsG* expression levels, various uninduced and induced strains were tested for αMG uptake (Table 3). $IICB^{Glc}$ activity in the wild type (LJ130) was induced about threefold by glucose, while LJ138 exhibited the expected constitutive transport activity. LZ1 and LJ132-3 showed increased and decreased uninduced transport activities, respectively. Both strains could be induced by the addition of glucose but, interestingly and in contrast to LJ130, also by the addition of glu-

TABLE 2. Phenotypes of various *ptsG* mutants from *E. coli* K-12 in the presence of different carbon sources[a]

Strain	Relevant mutation(s)	Phenotype in the presence of the following carbon source:				
		Glc	GlcN	Man	Rtl	Atl
LJ110	$ptsG^+$ $manXYZ^+$	+ (70)	+ (126)	+ (120)	r	r
LJ130 or LJ132	$ptsG^+$ Δ(*manXYZ*::*cat*)	+ (70)	−	−	r	r
LJ120	Δ(*ptsG*::*cat*) $manXYZ^+$	+ (125)	+ (126)	+ (120)	r	r
LZ1	$ptsG_1$ Δ(*manXYZ*::*cat*)	+ (72)	+ (280)	+ (144)	s	s
LJ132-3	$ptsG_3$ Δ(*manXYZ*::*cat*)	+ (72)	+ (210)	+ (125)	s	s
LJ138	$ptsG^+$ Δ(*manXYZ*::*cat*) *dgsA*::Tn*10kan*	+ (72)	−	−	r	r
HK727 (at 30°C)	$ptsG_{727}$ $manXYZ_{727}$ *ptsI19*(Ts) crr_{727}(Ts)	+	+	+	s	s
HK727 (at 42°C)		−	−	−	r	r
LZ727	$ptsG_{727}$ Δ(*manXYZ*::*cat*) pts^+ crr^+	+	+	+	s	s
JWL184-1	$ptsG_{184}$ $manXYZ_{184}$	+	−	+	r	r
JWL184-20	Δ(*ptsG*::*cat*)	−	−	−	r	r
JWL184-30	Δ(*manXYZ*::*cat*)	+	−	+	r	r

[a] Growth was tested on MacConkey indicator plates with 1% glucose (Glc), ribitol (Rtl), and D-arabinitol (Atl) or 0.5% mannose (Man) and on minimal agar plates with 0.2% glucosamine (GlcN). Reactions were classified as growth (+) and no growth (−) on minimal agar plates, as red (+, good fermentation) and colorless (−, no fermentation) colonies on MacConkey-glucose or MacConkey-mannose plates, and as sensitive (s) and resistant (r) colonies on MacConkey-ribitol or MacConkey-arabinitol plates. Generation times (in parentheses) determined in minimal medium with 0.2% carbohydrate are given in minutes. All cells were grown at 37°C except for HK727 cells, which were grown at 30 or 42°C as indicated.

TABLE 3. Induction by the uptake of αMG in isogenic strains[a]

Strain	Relevant mutation(s)	αMG uptake (nmol mg^{-1} min^{-1}) in the presence of the following inducer:				
		None	Glc	GlcN	Man	Rtl
LJ130	Δ(*manXYZ*::*cat*)	2.3	6.4	2.1	2.1	2.1
LJ138	*dgsA*::Tn*10kan*	9.8	5.0	9.3	7.7	8.4
LZ1	$ptsG_1$	6.5	12.1	16.6	11.4	12.9
LZ170	$ptsG_1$ *dgsA*::Tn*10kan*	15.8	15.1	19.2	15.8	20.3
LJ132-3	$ptsG_3$	1.1	6.4	6.6	6.2	5.2
LZ727	$ptsG_{727}$	22.6	20.1	22.5	21.4	18.5

[a] Cells were grown in liquid minimal medium with 0.2% glycerol plus a second carbohydrate (0.2%) as indicated and harvested during exponential growth. The uptake of αMG was tested at 25 μM (final concentration). The mean values of at least three measurements are given.

cosamine, mannose, or ribitol. The induced UmgC mutant LZ1 always exhibited higher transport activity than LJ130, thus resembling the $DgsA^-$ mutant LJ138. Introduction of the *dgsA*::Tn*10kan* mutation into LZ1 (producing strain LZ170) led to fully constitutive uptake activity which resembled the fully constitutive *ptsG* expression of LZ727 and that of other authentic UmgC strains, e.g., JM1110 (17). Interestingly, the induction levels in the wild type and the mutants LZ1 and LJ132-3 were always lower than those in the fully constitutive mutants LZ170 and LZ727.

The inducibility of *ptsG* expression in LZ1 was confirmed both by measuring the mRNA levels in cells growing either on glycerol or glucose (data not shown) and by monitoring the *ptsG* induction of both wild-type LZ110 and mutants LZ100 and LZ138 by use of the single-copy Φ(*ptsGop-lacZ*) fusion. At 15 min after the addition of glucose, in both wild-type LZ110 and $ptsG_1$ mutant LZ100, β-galactosidase activities started to increase, reaching a maximum at about 120 min after induction (Fig. 2). β-Galactosidase activities slowly decreased at the end of the exponential growth phase (data not shown). Basal LacZ activity was slightly higher in LZ100 but could be induced threefold, as in the wild-type strain. In the $DgsA^-$ mutant LZ138, maximum enzyme activity was high compared to those in LZ110 and LZ100; the addition of glucose caused a decrease in β-galactosidase activity probably as a consequence of a reduction in the intracellular cAMP level. The Φ(*ptsGop-lacZ*) fusion was also used to test for induction by substrates other than glucose (Table 4). Induction in the wild type occurred only after the addition of glucose, whereas *ptsG* expression in LZ100 was also induced by glucose, glucosamine, mannose, ribitol, D-fructose (Fru), and D-mannitol (Mtl) but not by the non-PTS carbohydrate L-arabinose (Ara).

The fusions were also used to test in our isogenic mutants, all derived from *E. coli* W3110, the influence of defined mutations known to affect *ptsG* expression (19, 37). The Δ*cyaA854* mutant LZ120 and the Δ(*ptsG*::*cat*) mutant LZ150 had low uninduced levels of β-galactosidase activities that could not be induced by any of the tested carbohydrates. This result implies that the induction of *ptsG* depends on the presence of cAMP-CrpA and of $IICB^{Glc}$, even though glucose can be transported by the II^{Man} system in a Δ(*ptsG*::*cat*) mutant, as shown by the ability to grow on glucose (Table 2). Introduction of a defined Δ(*ptsHI crr*::*kan*) mutation led to complete derepression of *ptsG* expression in LZ140, whereas the double mutant LZ160 [Δ(*ptsHI crr*::*kan*) Δ(*ptsG*::*cat*)] exhibited β-galactosidase activity that was below even the basal expression level in LZ150. Thus, intracellular glucose is not sufficient and the presence of nonphosphorylated $IICB^{Glc}$ is required for *ptsG* induction. Strains carrying the Δ(*dgsA*::*kan*) mutation exhibited constitutive β-galactosidase activity independently of the presence (LZ138) or absence (LZ139) of *ptsG*. These results confirm and extend data obtained by Plumbridge (38), who used strain JM101 and derivatives thereof for similar experiments (see Discussion).

Subcloning and sequencing of *ptsG* alleles from various $IICB^{Glc}$ mutants. All carbohydrates (Table 4) that induce *ptsG* expression in LZ1 have been postulated to be substrates of either wild type (40, 41) or mutant (3) $IICB^{Glc}$. We speculated that a mutation in this strain and in the other mutants changing the induction and substrate specificity of $IICB^{Glc}$ and not overexpression of the transporter per se was responsible for the UmgC phenotype. This hypothesis implied that there may be no distinct gene for a UmgC repressor protein, thus explaining our failure to detect a *umgC* gene in the vicinity of *ptsG*. To test this hypothesis, the *ptsG* alleles from all the mutants were amplified by a PCR. Additionally, strain JWL184-1, which was

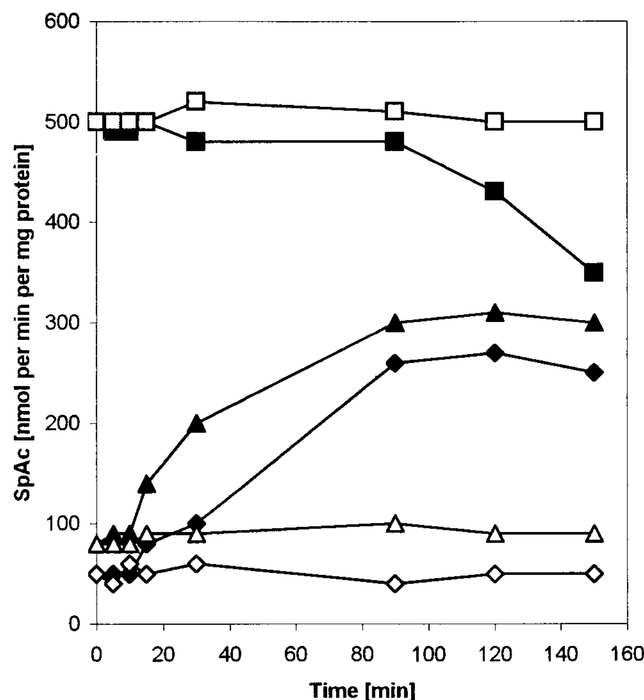

FIG. 2. Kinetics of induction of Φ(*ptsGop-lacZ*) in strains LZ110 (wild type), LZ100 ($ptsG_1$), and LZ138 (*dgsA*::*kan*). Cells harboring a single copy of the Φ(*ptsGop-lacZ*) translational fusion on F′8 were pregrown overnight in minimal medium with 0.2% glycerol and used to inoculate fresh medium with 0.2% glycerol. Glucose (0.2%) was added in the early exponential growth phase at 0 min. Samples were harvested and analyzed for β-galactosidase activity (SpAc). Symbols: ◇, uninduced LZ110; ◆, induced LZ110; △, uninduced LZ100; ▲, induced LZ100; □, uninduced LZ138; ■, induced LZ138.

TABLE 4. Induction tested by Φ(*ptsGop-lacZ*) expression in various isogenic mutants[a]

Strain	Relevant chromosomal mutation(s)	β-Galactosidase activity (nmol mg^{-1} min^{-1}) in the presence of the following inducer:							
		None	Glc	GlcN	Man	Rtl	Mtl	Fru	Ara
LZ110	*ptsG*$^+$	52	265	49	51	53	48	61	56
LZ100	*ptsG*$_1$	103	298	305	271	310	261	266	60
LZ120	Δ*cyaA854*	55	51	49	56	60	ND	ND	ND
LZ150	Δ(*ptsG*::*cat*)	48	52	47	50	50	ND	ND	ND
LZ140	Δ(*ptsHI crr*::*kan*)	295	280	278	302	290	ND	ND	ND
LZ160	Δ(*ptsG*::*cat*) Δ(*ptsHI crr*::*kan*)	30	29	27	32	25	ND	ND	ND
LZ138	Δ(*dgsA*::*kan*)	549	420	532	511	530	ND	ND	ND
LZ139	Δ(*ptsG*::*cat*) Δ(*dgsA*::*kan*)	450	450	ND	ND	ND	ND	ND	ND

[a] Various mutants derived from LJ110 (*ptsG*$^+$ *manXYZ*$^+$) and all carrying Φ(*ptsGop-lacZ*) on F′8 were tested for *ptsG* expression as transcribed from the wild-type *ptsG* promoter-operator. Cells were grown in minimal medium with 0.2% glycerol plus a second carbohydrate (0.2%) as indicated and harvested during exponential growth. The mean values of at least three measurements of β-galactosidase activities are given. ND, not determined.

described as being semiconstitutive for *ptsG* expression (23), was included in this approach. It exhibited a strong Glc$^+$ phenotype (55-min generation time) and weak Man$^+$ and Glm$^-$ Atl^r Rtl^r phenotypes. The introduction of defined *ptsG* or *manXYZ* mutations revealed that JWL184-1 carries a thus-far-unidentified *manXYZ* mutation and perhaps a *umgC*-like mutation in *ptsG* (Table 2).

Sequencing analysis showed that each mutant carried a single-base-pair substitution which caused an amino acid exchange (Table 5). Interestingly, the same Ser169 is replaced either by a Phe residue in LZ1 or by a Pro residue in LJ132-3. The mutation Glu387Gly in the HK727 *ptsG* allele is located in the putative linker region between the IIC and IIB domains of the glucose permease (Fig. 3), whereas the mutation Phe195Leu in JWL184-1 appears to be located in putative transmembrane helix 6 of the IIC domain (5).

For a complementation analysis of the various *ptsG* alleles, the genes were cloned and expressed as *Pst*I/*Hin*dIII fragments in pTM30 (Fig. 1). The IICB^{Glc} quantities expressed from these plasmid constructs without induction were as high as those in induced wild-type LJ110, as indicated by Western blot analysis with a polyclonal antiserum against the IIB^{Glc} domain (kindly provided by B. Erni). The addition of at least 50 μM IPTG led to complete growth inhibition (data not shown).

The various *ptsG* alleles were able to complement the *ptsG manXYZ* double mutant LJ121 to a positive phenotype on MacConkey-Glc plates. However, only the mutated IICB^{Glc} transporters expressed from pTM1, pTM32-3, and pTM727 produced the typical UmgC phenotype shown by their parent strains LZ1, LJ132-3, and LZ727 (Table 2) and were transdominant over *ptsG*$^+$, e.g., in LJ130 (data not shown). LJ121 harboring pTM184 (from JWL184-1) became Glc$^+$ and Man$^+$ but remained Glm$^-$ Atl^r Rtl^r. These results were further confirmed by measuring the pattern of induction of Φ(*ptsGop-lacZ*) expression in LZ150/F′8::Tn Φ(*ptsGop-lacZ*) carrying the various *ptsG* alleles on pTM30. In the presence of the wild-type allele on pTM110, only glucose (2.5-fold) and perhaps mannose (1.7-fold) produced induction (Table 5). The mutated transporters expressed from pTM1 and pTM32-3 also caused inducibility by glucosamine, mannose, ribitol, mannitol, and fructose but not by the non-PTS carbohydrate arabinose, whereas cells harboring pTM184 were significantly induced by glucose (4-fold) and mannose (2.6-fold). Moreover, by expression of the original UmgC mutant allele from pTM727, constitutive β-galactosidase activity was observed.

These results corroborate the hypothesis that altered IICB^{Glc} from LZ1, LJ132-3, and HK727 alone is responsible for the typical UmgC phenotype and that no additional mutation is involved. JWL184-1 carries a different type of *ptsG* mutation. The substrate specificity of this mutated IICB^{Glc} is less relaxed than that of the transporters from the other mutants in that it results in only enhanced mannose transport. It is important to note that the two newly isolated mutations from LZ1 and LJ132-3 and the one from JWL184-1 differ from the HK727 *ptsG* allele in that they do not cause transdominant constitutive *ptsG* expression.

Rtl^r derivatives of LZ1 and LJ132-3 were isolated on MacConkey agar plates with 1% ribitol. The majority (93%) of these either had *ptsG* completely deleted or inactivated by IS*10* insertions or carried other mutations in *ptsG* (Glc$^-$ Man$^-$ Glm$^-$ phenotype), as revealed by Southern hybridization and DNA sequencing analysis (data not shown), confirming that IICB^{Glc} is the only transport system for these carbohydrates in our isogenic strains. Others (4%) had a pleiotropic negative phenotype for PTS carbohydrates, resembling PtsI$^-$ and PtsH$^-$ mutants (data not shown). Inactivation of *ptsHI crr*

TABLE 5. Influence of various *ptsG* alleles on *ptsG* induction as measured in LZ150 by the F′8::Tn Φ(*ptsGop-lacZ*) test system[a]

Plasmid	Allele	Mutation	Amino acid exchange	β-Galactosidase activity (nmol mg^{-1} min^{-1}) in the presence of the following inducer:							
				None	Glc	GlcN	Man	Rtl	Mtl	Fru	Ara
pTM30	Control			70	70	77	80	88	89	99	90
pTM110	*ptsG*$^+$			75	202	62	125	83	74	99	98
pTM1	*ptsG*$_1$	TCC→TTC	S169F	72	212	298	297	175	199	166	101
pTM32-3	*ptsG*$_3$	TCC→CCC	S169P	75	206	277	258	194	165	175	111
pTM727	*ptsG*$_{727}$	GAA→GGA	E387G	492	226	528	398	537	317	414	406
pTM184	*ptsG*$_{184}$	TTT→CTT	F195L	67	262	92	177	58	104	110	80

[a] LZ150 Δ(*ptsG*::*cat*) *man*$^+$/F′8::Tn Φ(*ptsGop-lacZ*) harboring various plasmids was grown in liquid minimal medium with 0.2% glycerol, 0.1% Casamino Acids, and 50 mg of ampicillin per liter. For induction, a second carbohydrate (0.2%) was added, and cells were harvested during exponential growth. The mean values of at least three measurements of β-galactosidase activities are given.

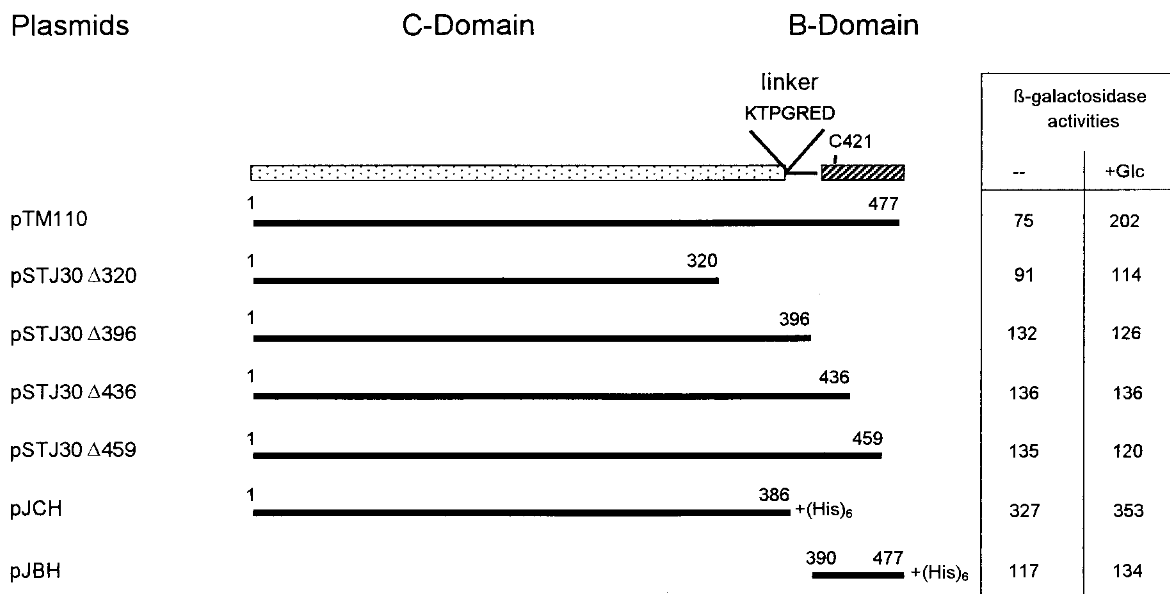

FIG. 3. Construction of carboxy-terminal deletions of IICB^{Glc} and testing of *trans*-activation of a Φ(*ptsGop-lacZ*) fusion. The functional domains of IICB^{Glc} are indicated as follows: the amino-terminal, hydrophobic IIC^{Glc} domain is shown as a stippled box; the carboxy-terminal, hydrophilic IIB^{Glc} domain with the phosphorylated Cys421 residue is shown as a hatched box; and the linker with the conserved 382-KTPGRED-388 motif is shown as a thin line. Deletion plasmids are shown as thick lines, and the associated numbers refer to the first and the last original amino acids of the truncated IICB^{Glc} protein. Plasmids pJCH and pJBH (kindly provided by B. Erni) encode six additional His residues. LZ150/F′8::Tn Φ(*ptsGop-lacZ*) harboring various plasmids was grown in liquid minimal medium with 0.2% glycerol, 0.1% Casamino Acids, and 10 mg of ampicillin per liter. Glucose (0.2%) was added after one cell division cycle where indicated (+Glc). Cells were harvested during exponential growth. β-Galactosidase activities are given as nanomoles of protein per milligram per minute. The mean values of at least two measurements are given.

probably occurred less frequently than inactivation of *ptsG* because of the reduced viability of mutants with a lesion in general PTS proteins.

Three Rtl^r derivatives, LZ22 and LZ23 from LZ1 and LJ333 from LJ132-3, with a Glc$^+$ Man$^-$ Glm$^-$ phenotype, were further characterized. DNA sequencing analysis of both *ptsG* promoter-operator regions and reading frames revealed that in each case, the original mutation was still present. However, LZ22 showed one base-pair substitution, a T-to-G transversion (boldface), in the previously identified −10 region (TTTAC TCT to TT**G**ACTCT) (19, 37); this change most likely leads to a strong reduction in *ptsG* expression. The *ptsG* allele of LZ23 carried an additional Thr246Ser amino acid exchange, whereas LJ333 had additional Ala82Pro and Val83Ile exchanges in IICB^{Glc}. These additional mutations apparently reduce transport activity.

Effects of plasmid-encoded and truncated IICB^{Glc} on the level of expression of a Φ(*ptsGop-lacZ*) fusion in a Δ(*ptsG::cat*) strain. Deletion of *ptsG* completely prevented the induction of a Φ(*ptsGop-lacZ*) fusion by the addition of glucose, even in a Man$^+$ strain, or any other tested carbohydrate (Table 4). We previously reported (T. Zeppenfeld, C. Larisch, J. W. Lengeler, and K. Jahreis, Abstr. Int. Meet. Fachgr. Biochem. GDCh and SF431, p. 68, 1999) that IIC^{Glc} expression in the presence of pJCH alone (6), which encodes the IIC^{Glc} domain and parts of the linker region (amino acids 1 to 386 plus six additional His residues), resulted in strong constitutive β-galactosidase activity (Fig. 3) in LZ150/F′8::Tn Φ(*ptsGop-lacZ*). The truncated protein was not capable of transporting glucose; i.e., there was no complementation for glucose uptake in the *ptsG manXYZ* double mutant LJ121 (data not shown). Plumbridge (38) suggested that the IIB^{Glc} domain of PtsG may be responsible for generating the inducing signal for *ptsG* expression. Therefore, we also tested plasmid pJBH (6), which encodes the IIB^{Glc} domain (amino acids 390 to 477 plus six additional His residues), for transactivation in this assay. Cells harboring pJBH exhibited only slightly, if any, increased β-galactosidase activity from the Φ(*ptsGop-lacZ*) fusion compared to the wild-type control. These results correspond to the results obtained by Plumbridge (38), who also observed only a very weak positive regulatory effect of overproduction of the IIB^{Glc} domain.

To further investigate what part of the IICB^{Glc} domain might be responsible for the interaction with DgsA, we constructed different plasmids that encode proteins with defined carboxy-terminal deletions within the IIB^{Glc} domain (Fig. 3). The addition of 50 μM IPTG to cells harboring these plasmid constructs led to immediate growth arrest, providing some evidence for the production of the truncated proteins. However, attempts to detect these truncated proteins by Western blot analysis with a polyclonal antiserum against the hydrophilic IIB^{Glc} domain failed. None of these deletion plasmids could complement for glucose uptake, e.g., in LJ121; however, like pJBH, all pSTJ30 derivatives slightly increased β-galactosidase activity from a Φ(*ptsGop-lacZ*) fusion in LZ150, compared to the uninduced wild type or the pTM30 control (Fig. 3 and Table 5). This increased β-galactosidase activity was independent of the addition of glucose.

Effects of mutated IICB^{Glc} from LZ1 on the level of expression of a Φ(*ptsHp-lacZ*) fusion. Besides regulating *ptsG* expression, DgsA is also involved in the regulation of the *pts* operon (18, 38). To test whether the *ptsG*$_1$ allele has an effect on the *pts* operon, a single-copy Φ(*ptsHp-lacZ*) operon fusion was constructed. In wild-type *ptsG*$^+$ strain LZ110, *ptsH* expression was induced about 3.3-fold by the addition of glucose (Table 6), slightly by mannose and mannitol, but not at all by ribitol. In the *ptsG*$_1$ mutant LZ100, the induction pattern changed as described for the Φ(*ptsGop-lacZ*) fusion; i.e., the basal expres-

TABLE 6. Induction of Φ(*ptsHp-lacZ*) expression in LZ110 (*ptsG*$^+$) and LZ100 (*ptsG*$_1$) by different carbon sources[a]

Strain	β-Galactosidase activity (nmol mg^{-1} min^{-1}) in the presence of the following inducer:				
	None	Glc	Man	Rtl	Mtl
LZ110 Φ(*ptsHp-lacZ*)	100	330	140	100	140
LZ100 Φ(*ptsHp-lacZ*)	150	400	510	310	280

[a] LZ110 (*ptsG*$^+$) and LZ100 (*ptsG*$_1$) harboring F′8::Tn Φ(*ptsHp-lacZ*) were grown in minimal medium with 0.2% glycerol plus a second carbohydrate (0.2%) as indicated and harvested during exponential growth. The mean values of at least two measurements of β-galactosidase activities are given.

sion level and the level of expression with glucose were increased, and the mutant was clearly inducible by the addition of mannose, ribitol, and mannitol (three- to fivefold). These changes, caused by alteration of the IICB^{Glc} transporter, are consistent with the hypothesis that the substrate binding and/or transport activities of IICB^{Glc} directly modulate DgsA binding activity for all DgsA-regulated operons (see Discussion).

DISCUSSION

In this study, we were able to show the mechanism of previously described *umuC* mutations in *E. coli* K-12 (17). We provide evidence for the nonexistence of a postulated distinct *umgC* regulatory gene for *ptsG* expression and show that mutations causing the UmgC phenotype map within *ptsG*. From the analysis of existing and newly isolated UmgC mutants emerges a novel regulatory model for *ptsG* expression. It contains, besides classical regulatory proteins, new elements of a glucose sensory system which correspond to parts of the glucose PTS. Essential components of this new model have been described by Kimata et al. (19) and by Plumbridge (38).

Central regulatory elements for the *ptsG* promoter-operator are the repressor DgsA (also known as Mlc), the PTS-dependent phosphorylation state of the IICB^{Glc} complex, and the global cAMP-CrpA activator. As has been shown by direct in vitro binding assays, *ptsG* transcription is repressed by DgsA binding to the operator and activated by cAMP-CrpA (19, 37). In accordance with the new model, the expression of IICB^{Glc} cannot be induced above the basal level in a Δ*cyaA* background, whereas it is constitutively high in a Δ*dgsA cyaA*$^+$ background (Table 4). No molecular inducer for DgsA could be found in gel retardation assays which included glucose and glucose-6-phosphate (18, 19, 37). The new model postulates that IICB^{Glc} is the glucose sensor responsible for the induction of *ptsG* and that IIC^{Glc} occurs in two conformations: one (IIC) with a low affinity and the other (IIC*) with a high affinity for DgsA. IIC* prevents DgsA from binding to the operator, either by competition for a limited amount of DgsA or by allosterically reducing the affinity of DgsA for the operator. In the absence of IICB^{Glc} (in a Δ*ptsG* strain), DgsA remains permanently bound to its operator, resulting in complete repression of transcription. In the absence of the DgsA repressor (in a Δ*dgsA* strain), transcription becomes constitutive, independent of the presence of IICB^{Glc} but still dependent on activation by cAMP-CrpA. We speculate that phosphorylated IIB^{Glc} either shifts the equilibrium toward the low-affinity IIC^{Glc} conformation or that phosphorylated IIB^{Glc} binds to the IIC^{Glc} domain and thereby excludes DgsA from binding. As predicted by the model, mutants with defects in EI (*ptsI*), HPr (*ptsH*), or IIA^{Glc} (*crr*) all show constitutive expression of *ptsG*, provided an intact or a mutated IICB^{Glc} complex is also present (Table 4). Thus, Δ*ptsG* Δ*pts* mutants are noninducible. Final support for the model comes from the observation that mutants expressing DgsA and the IIC^{Glc} domain in the absence of the IIB^{Glc} and/or IIA^{Glc} domain, i.e., the putative IIC* state, show constitutive *ptsG* expression (Table 4 and Fig. 3). The failure of the C-terminal deletion proteins of IICB^{Glc} encoded by the pSTJ30 deletion derivatives (Fig. 3) to generate (like pJCH) enhanced constitutive *ptsG* expression might be caused by a permanent "locked-off" conformation of the IIC^{Glc} domain or by reduced protein stability.

In contrast to the *bgl* and other operons controlled through a PTS-dependent antitermination system (reviewed in reference 51), no antiterminator protein is involved in *ptsG* control in *E. coli*, and there is no evidence thus far for PTS-dependent phosphorylation or dephosphorylation of either the repressor DgsA (18) or the global activator CrpA. This observation again corroborates the new model in which the PTS-dependent phosphorylation of IICB^{Glc} acts through alteration of the conformation of the inducer domain IIC^{Glc} rather than through phosphotransfer to a cognate regulator. Based on the new model, the UmgC and other *ptsG* mutations can now be understood in the following way.

(i) The fully constitutive allele *ptsG*$_{727}$ from the original HK727 mutant is a "locked-in" mutation which blocks IIC permanently in the IIC* state (Table 5). Moreover, all mutated *ptsG* alleles exhibit *trans*-dominance over the wild-type *ptsG* allele. The corresponding E387G mutation affects a linker motif postulated before as being essential for the communication between IIB^{Glc} and IIC^{Glc} (5, 6, 24). In *E. coli*, there is no efficient ATP-dependent glucosamine kinase which could generate the first obligatory intermediate, glucosamine-6-phosphate. Furthermore, glucosamine, normally transported and phosphorylated through II^{Man}, is a noninducing and poor substrate for IICB^{Glc}. Therefore, selecting for Glm$^+$ derivatives in a ManA$^-$ mutant selects for mutations allowing a high level of expression of IICB^{Glc} and efficient phosphorylation of glucosamine by the glucose PTS. Thus, the constitutive DgsA mutant (LJ138 in Table 2) remains unable to take up and phosphorylate this substrate efficiently, while all UmgC-like mutants accept this substrate fairly well. In contrast, other mutants are able to grow on D-ribose and D-xylose via the IICB^{Glc} transporter but in the absence of concomitant substrate phosphorylation (33). Such mutations always arise in combination with a *dgsA* mutation, indicating that substrates transported through IICB^{Glc} by facilitated diffusion do not induce *ptsG* due to the lack of dephosphorylation of IIB^{Glc} during the process.

(ii) Besides the fully constitutive type of UmgC mutations represented by *ptsG*$_{727}$, there are semiconstitutive and inducible UmgC mutations; i.e., they require the presence of a substrate which is taken up and phosphorylated by the IICB^{Glc} transporter for full induction. They range from efficiently accepting all four substrates and having a clearly increased (≥2-fold) basal induction level (S169F) to allowing only glucosamine (P238L; data not shown) or mannose (F195L) and not arabinitol and ribitol transport and phosphorylation. Similar UmgC mutations, but in different amino acid residues (G176D, A288V, G320S, and P384R), have been isolated by Notley-McRobb and Ferenci (31). It is tempting to speculate that all of these mutations lead to a relaxed substrate specificity of the IICB^{Glc} transporter. In accordance with this hypothesis, this group isolated another class of UmgC-like mutants (V12G, V12F, and G13C) with increased growth on glucosamine and enhanced growth on glucose and mannose (31).

It is more difficult to explain how mutations (boldface) in the hydrophilic linker motif (382-K**T**P**G**R**E**D-388) cause changes in substrate specificity relative to mutations located in the

membrane-bound IIC^{Glc} domain, which is supposed to carry central parts of the substrate binding and catalytic site. The function of this interdomain linker in transport was systematically investigated by alanine-scanning mutagenesis (20). Amino acid substitutions T383A and G385A caused a strong reduction in phosphotransfer activity. The linker perhaps plays an important role in the conformational changes of the protein, precisely coupling the interaction between the IIB^{Glc} and the IIC^{Glc} domains in the process of phosphotransfer to the substrate bound in IIC^{Glc} (5, 6, 24). Substantial changes in the conformation of a PTS transporter during the process of substrate binding and phosphorylation have been reported for IICBA^{Mtl} (28). Using isothermal titration calorimetry, these authors found that approximately 50 to 60 residues are involved in the binding and phosphorylation of the substrate mannitol and the interaction between IIC^{Mtl} and IIB^{Mtl}. This interaction seems to be necessary for phosphotransfer from IIB^{Mtl} to the IIC^{Mtl}-bound substrate and the release of the phosphorylated substrate into the cytoplasm. The fact that the E387G mutant exhibits altered substrate specificity, enhanced transport activity, and a locked-in conformation for the interaction with DgsA seems to indicate that substrate binding, dephosphorylation of IIB^{Glc}, and a change into the inducing conformation (IIC*) are related processes. The observation that the S169F mutation caused an increased level of basal expression also fits this hypothesis.

The unexpected finding that our laboratory strain JWL184-1 carries a mutation in *ptsG* indicates that other laboratory strains of *E. coli* K-12 also may carry unidentified *ptsG* mutations. Plumbridge (38), for example, reported that in her JM101 copy, a Φ(*ptsGop-lacZ*) fusion was inducible by glucose, *N*-acetylglucosamine, mannitol, trehalose and, to a lesser extent, glucosamine and mannose. This finding is in contrast to our finding that the *E. coli* K-12 reference strain W3110 and its isogenic derivatives can be induced only by glucose. Characteristically, older transport studies had indicated D-glucose, αMG, and 5-thio-D-glucoside as the only substrates for the IICB^{Glc} transporter (39).

An interesting question is how DgsA could be titrated by IICB^{Glc} if the *dgsA* gene itself is autoregulated. DgsA binding sites cloned on low-copy-number plasmids indeed do not titrate DgsA and therefore do not lead to constitutive expression of DgsA-controlled genes (unpublished results). Cloning of the same DgsA binding sites on a multicopy-number vector, however, led to titration of DgsA and to constitutive Φ(*ptsGop-lacZ*) expression (38). This result indicates that *dgsA* expression is rather limiting and that titration of DgsA by IICB^{Glc} present at about 2,000 molecules per cell might be possible.

The results presented here extend and contribute to understanding of the function of IICB^{Glc} not only in glucose transport but also in glucose sensing and response. In this process, the transport of glucose triggers a dual-signal transduction pathway. One branch consists of the glucose-dependent modulation of the level of phosphorylation of IIA^{Glc}. Unphosphorylated IIA^{Glc} binds to and reversibly inhibits non-PTS transporters, e.g., for lactose, maltose, or glycerol (inducer exclusion); in its phosphorylated form, IIA^{Glc} activates adenylate cyclase to synthesize cAMP (reviewed in reference 22). In the second branch, the level of phosphorylation of IICB^{Glc} directly modulates the activity of the anticatabolite repressor DgsA. DgsA, in interaction with cAMP-CrpA, can be used by *E. coli* to precisely regulate carbon catabolite gene expression.

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