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Positive Regulation of the *pts* Operon of *Escherichia coli*: Genetic Evidence for a Signal Transduction Mechanism

H. DE REUSE* AND A. DANCHIN

Unité Régulation de l'Expression Génétique, Institut Pasteur, 28, rue du Docteur Roux, 75724 Paris Cedex 15, France

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The *pts* operon of *Escherichia coli* is composed of the genes *ptsH*, *ptsI*, and *crr*, which code for three proteins of the phosphoenolpyruvate-dependent phosphotransferase system (PTS): the HPr, enzyme I (EI), and $EIII^{Glc}$ proteins, respectively. These three genes are organized in a complex operon in which the major part of expression of the distal gene, *crr*, is initiated from a promoter region within *ptsI*. Expression from the promoter region of the *ptsH* and *ptsI* genes has been studied in vivo by using gene fusions with *lacZ*. Transcription from this promoter region is under the positive control of catabolite activator protein (CAP)-cyclic AMP (cAMP) and is also enhanced during growth in the presence of glucose (a PTS substrate). This report describes a genetic characterization of the mechanism by which growth on glucose causes transcriptional stimulation of the *pts* operon. This regulation is dependent on transport through the glucose-specific permease of the PTS, EII^{Glc}. Our results strongly suggest that transcriptional regulation of the *pts* operon is the consequence of an increase in the level of unphosphorylated EII^{Glc} which is produced during glucose transport. Furthermore, overproduction of EII^{Glc} in the absence of transport was found to stimulate expression of the *pts* operon. We also observed that CAP-cAMP could cause stimulation independently of the EII^{Glc} and that glucose could activate in the absence of cAMP in a strain overproducing EII^{Glc}. Our results indicate that glucose acts like an environmental signal through a mechanism of signal transduction. A sequence similarity between the C terminus of EII^{Glc} and the consensus of transmitter modules of the sensor proteins defined by E. C. Kofoid and J. S. Parkinson (Proc. Natl. Acad. Sci. USA 85:4981–4985, 1988) suggests that EII^{Glc} might have properties in common with the sensors of the two-component systems.

The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) catalyzes uptake and phosphorylation of a large number of carbohydrates in many different bacteria (for reviews, see references 19, 20, and 22). This system involves sequential phosphoryl group transfer events. The phosphoryl group of the donor molecule, PEP, is transferred to a series of PTS proteins and finally to the transported PTS carbohydrate. In *Escherichia coli*, two of these proteins are common to almost all PTS carbohydrates. Other proteins (at least 20) are specific for one or a few PTS carbohydrates. The two common proteins, HPr and enzyme I (EI), encoded by the *ptsH* and *ptsI* genes, respectively, are cytoplasmic. The specific PTS proteins are either cytoplasmic phosphoproteins or membrane-bound permeases.

Glucose is one of the PTS carbohydrates. Its transport requires, in addition to EI and HPr, either of two other sets of specific proteins. The major part of glucose transport is mediated by the cytoplasmic enzyme $EIII^{Glc}$, encoded by the *crr* gene, and the glucose-specific permease, EII^{Glc}, encoded by the *ptsG* gene. EII^{Glc} is phosphorylated on a His residue by $EIII^{Glc}$ (2, 18). During transport, phosphorylated EII^{Glc} ($P\text{-}EII^{Glc}$) transfers its phosphoryl group to glucose, becomes dephosphorylated, and releases intracellular glucose-6-phosphate. Glucose can also be transported through another PTS pathway which was defined as the mannose PTS involving $EIII^{Man}$ and the EII^{Man} enzymes (10, 32).

The *ptsH*, *ptsI*, and *crr* genes are linked at 52 min on the *E. coli* chromosome. The order of the genes at this locus is *cysK-ptsH-ptsI-crr* in the clockwise orientation. The *ptsG* gene encoding EII^{Glc} does not map together with the *crr* gene but rather at 25 min. This organization is different from that of the genes encoding the other EII-EIII pairs, which are always linked (summarized in reference 6). The nucleotide sequences of the *ptsH*, *ptsI*, and *crr* genes have been determined (5, 25). The *cysK* gene was also sequenced, and its promoter was identified (4, 13). We have previously analyzed the transcription of the *ptsH*, *ptsI*, and *crr* genes. These three genes are cotranscribed as an operon with a promoter region located upstream from *ptsH* and a rho-independent terminator located downstream from *crr*. Transcription at the *pts* operon is organized in a more complex manner, since the proximal and the distal genes are also expressed from two short gene-specific transcripts. The *ptsH*-specific transcript is probably due to an early termination event within the *ptsI* gene, whereas approximately 80% of *crr* expression is due to transcription from a strong promoter region located within *ptsI* (5).

The cellular concentrations of the two common PTS proteins, HPr and EI, are the predominant factors which determine the phosphorylation levels of all PTS proteins and therefore affect the efficiency of transport of all PTS carbohydrates. In addition, it was observed that the phosphorylated form of $EIII^{Glc}$ activates adenylate cyclase, the enzyme catalyzing cyclic AMP (cAMP) synthesis (28). The intracellular concentration of cAMP was shown to determine the level of expression of many catabolite genes or operons by regulating their transcription when complexed to its receptor protein, catabolite activator protein (CAP) (33). In addition, $EIII^{Glc}$, when unphosphorylated, is able to inhibit a number of non-PTS transport systems (29). As pointed out by Roseman and Meadow (22), the ratio of $P\text{-}EIII^{Glc}$ to $EIII^{Glc}$ is the key element in the PTS-mediated regulation of carbohydrate utilization.

The levels of HPr, EI, and $EIII^{Glc}$ under different growth conditions were determined by Rephaeli and Saier (21),

* Corresponding author.

TABLE 1. *E. coli* strains used

Strain	Genotype	Origin, source, or reference
TP2100	F⁻ *xyl argH1 ilvA lacΔX74*	Roy et al. (24)
TP2110	F⁻ *xyl argH1 ilvA lacΔX74 recA srl*::Tn*10*	De Reuse et al. (7)
TP2006	F⁻ *xyl lacΔX74 Δcya glp8306*	Roy and Danchin (23)
TP2504	F⁻ *xyl argH1 ilvA zcf-229*::Tn*10 ptsG22*	De Reuse and Danchin (5)
TP2511	F⁻ *xyl argH1 zcf-229*::Tn*10 ptsG22 Δcya*	Phage P1(TP2006) transductant of strain TP2504
TP2512	F⁻ *xyl argH1 zcf-229*::Tn*10 ptsG22 Δcya glp8306*	Phage P1(TP2006) transductant of strain TP2511
TP2811	F⁻ *xyl argH1 aroB ilvA lacΔX74 Δ(ptsH-ptsI-crr)* Km^r	Lévy et al. (14)
TP2508	F⁻ *xyl argH1 zcf-229*::Tn*10 ptsG22 Δ(ptsH-ptsI-crr)* Km^r	Phage P1(TP2811) transductant of strain TP2504
TP2513	F⁻ *xyl lacΔX74 Δcya glp8306 Δ(ptsH-ptsI-crr)* Km^r	Phage P1(TP2811) transductant of strain TP2006
LM1	F⁻ *argG6 crr galT his-1 manA manI metB nagE rpsL thi*	Lengeler et al. (12)
TP2823	F⁻ *xyl argH1 ilvA lacΔX74 Δ(ptsH-ptsI-crr)* Tet^r	S. Lévy, unpublished strain constructed as in reference 14
TP2505	F⁻ *xyl argH1 ilvA lacΔX74 crr*	Phage P1(LM1) transductant of strain TP2823
TP2862	F⁻ *xyl argH1 ilvA aroB Δ(crr)* Km^r	Lévy et al. (14)
IBPC542	F⁻ *thi-1 argG6 argE3 his-4 mtl-1 xyl-5 tsx-29 rpsL lacΔX74 nagE*::Kan^r	J. Plumbridge (unpublished)
TP2509	F⁻ *xyl argH1 ilvA lacΔX74 crr nagE*::Kan^r	Phage P1(IBPC542) transductant of strain TP2505
R1360	*ara Δ(lac-pro) strA thi Δbgl*::Tet^r	K. Schnetz and B. Rak (unpublished)
TP2514	F⁻ *xyl argH1 ilvA lacΔX74 crr nagE*::Kan^r *Δbgl*::Tet^r	Phage P1(R1360) transductant of strain TP2509

Stock et al. (32), and Mattoo and Waygood (15). We performed an extensive study of the regulation of expression of the *ptsH*, *ptsI*, and *crr* genes under different growth conditions (5). Expression of these genes can be defined as semiconstitutive. In fact, although the basal level of expression of the *pts* operon is appreciable, we found that its level of expression was modulated by at least two factors (described below). We showed, using gene fusions with *lacZ*, that this transcriptional regulation occurred at the level of the *ptsH-ptsI* promoter region. Expression of the *crr* gene is barely modulated, since transcription from its own promoter remained unchanged under the various growth conditions tested (5).

The two factors regulating expression of the *ptsH* and *ptsI* genes are the nature of the sugar and the cAMP level in the cell. We showed that transcription from the *ptsH-ptsI* promoter region is stimulated during growth in the presence of glucose and that it is under the positive control of the CAP-cAMP complex (5).

This report describes a genetic characterization of the mechanism by which growth on glucose causes positive regulation at the *pts* operon. This regulation implies a mechanism of signal transduction. Indeed, stimulation of *pts* operon expression is dependent on transport through the glucose-specific permease of the PTS, EII^{Glc}. We show that an increase in the level of the unphosphorylated form of EII^{Glc}, which is produced during transport of glucose through this permease, generates, directly or indirectly, transcriptional regulation at the *pts* operon. The role of unphosphorylated EII^{Glc} is further emphasized by the observation that overproduction of EII^{Glc}, in the absence of transport, causes stimulation of *pts* operon expression. We finally observed that CAP-cAMP is able to activate expression of the *pts* operon independently of EII^{Glc} and that glucose can stimulate this operon in the absence of cAMP if EII^{Glc} is in excess. Various pieces of evidence suggest there is an analogy between this signal transduction mechanism and the so-called two-component regulatory systems (31).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains used are listed in Table 1. Bacteriophage P1 transduction (16) was used in the construction of strains when required. All strains used for the β-galactosidase assays are derivatives of the same original strain (TP2100) and therefore have closely related genetic backgrounds. Growth media were either rich medium LB or synthetic medium M63 supplemented with a carbon source (0.4%), thiamine (10 μg/ml), the required amino acids (100 μg/ml), and 0.1% Casamino Acids (Difco Laboratories) (16). cAMP was added at a final concentration of 3 mM. Antibiotic concentrations were 50 μg/ml for ampicillin and 25 μg/ml for kanamycin. Screening for the ability to synthesize β-galactosidase was performed on MacConkey plates containing 1% lactose or on M63 lactose plates. Measurements of PEP-dependent phosphorylation of [^{14}C]methyl-α-glucoside transported with high affinity via the EIII^{Glc}-EII^{Glc} enzymes were performed on toluene-treated cells under the conditions described by Bouvet and Grimont (3). PEP-dependent phosphorylation activities in the *pts* mutant strains compared with that of the *pts*$^+$ strain TP2110 were as follows: TP2504 (*ptsG*), 1%; TP2811 [*Δ(ptsH ptsI crr)*], 5%; TP2505 (*crr*), 11%; and TP2862 (*Δcrr*), 19%. The TP2512 (pDIA3247, PTSG10) strain (Fig. 2) was constructed as follows. Strain TP2512 (*Δcya ptsG*) was transformed with plasmid PTSG10, which carries the *ptsG* gene (a generous gift of B. Erni; 9) and generated stable colonies which had recovered the ability to grow on glucose. Strain TP2512 containing plasmid PTSG10 was then transformed by the compatible plasmid carrying the *ptsH-lacZ* fusion, pDIA3247.

β-Galactosidase assays. β-Galactosidase was assayed by the method of Pardee et al. (17); 1 U was defined as the amount of enzyme that converted 1 nmol of substrate per min at 28°C. The values indicated in Tables 2 and 3 and in Fig. 2 and 3 correspond to β-galactosidase rates of synthesis, $\Delta Z/\Delta B$. Each rate of synthesis was calculated from β-galactosidase assays on approximately 10 samples. These samples were withdrawn during exponential growth at 37°C in synthetic medium M63 supplemented as indicated above, in the figure legends, and in the tables. Each experiment was reproduced at least two times. We calculated the linear regression value R for each $\Delta Z/\Delta B$ curve. On the 29 $\Delta Z/\Delta B$ values of the tables and the figures, 11 had an R value of 1, 14 had an R value of 0.99, 3 had an R value of 0.98, and 1 had an R value of 0.96. In addition, we calculated the 95% confidence limits and indicated them for each $\Delta Z/\Delta B$ value in parentheses in the tables and figures. These values were

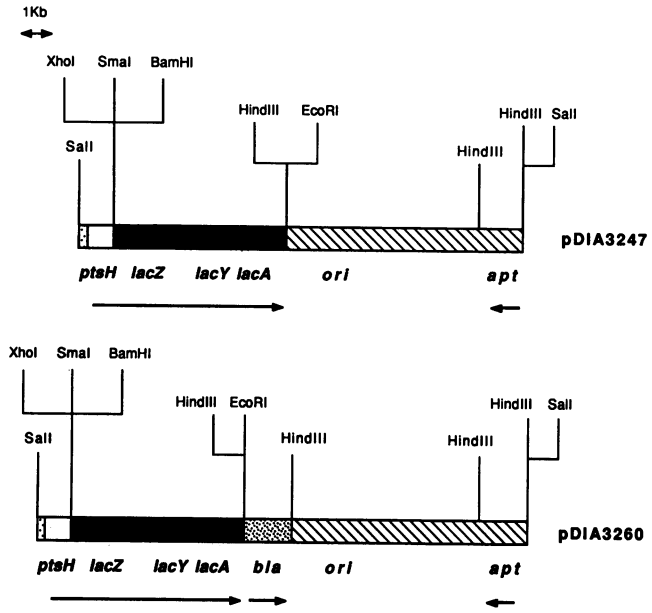

FIG. 1. Restriction map of plasmids pDIA3247 and pDIA3260 carrying protein fusions between the *ptsH* gene and the *lacZ* gene. ▨, Vector pSA206; ▩, fragments of pBR322; *apt*, gene conferring kanamycin resistance; *bla*, gene conferring ampicillin resistance; *ori*, origin of replication. Direction of transcription of the genes is indicated by arrows. For construction of these plasmids, see Materials and Methods.

calculated as 2σ values, where σ is the standard deviation. The β-galactosidase rates of synthesis are expressed in units per milligram (dry weight) of bacteria, deduced from the optical density at 600 nm, considering that 1 mg (dry weight) per ml is estimated to be 3.7 optical density units at 600 nm.

Plasmids. The two plasmids used, pDIA3247 and pDIA3260, are illustrated in Fig. 1. Both carry the identical protein fusion between *ptsH* and *lacZ* on the low-copy-number vector pDIA3231 (7). This vector belongs to the IncW incompatibility group, and we showed previously (7) that its copy number remains unchanged in growth conditions identical to those of the experiments described here: growth on glucose as well as growth in a Δ*cya* strain with or without cAMP. Plasmid pDIA3247 has been described by De Reuse and Danchin (5). Plasmid pDIA3260 is a derivative of pDIA3247 constructed by insertion of a *Hin*dIII restriction fragment carrying the complete *bla* gene encoding β-lactamase. Plasmid pDIA3260 thus contains the gene for kanamycin resistance as well as the gene for ampicillin resistance. This latter plasmid was used because four strains, TP2862, TP2508, TP2513, and TP2811, already carry the gene conferring kanamycin resistance.

Reagents and enzymes. T4 DNA ligase and restriction enzymes were used as recommended by the suppliers (Boehringer Mannheim, Pharmacia, Amersham, and Appligène). DNA fragments were analyzed by standard gel electrophoresis (0.5 to 1% agarose; Tris-borate buffer) and ethidium bromide staining (30). Agarose was from Bethesda Research Laboratories; ampicillin, kanamycin, methyl-α-glucoside, and *o*-nitrophenyl-β-D-galactopyranoside were from Sigma or Boehringer Mannheim; and other chemicals were from Merck. [^{14}C]methyl-α-glucoside was obtained from Amersham.

RESULTS

Glucose-dependent regulation of the *pts* operon necessitates translocation through EII^{Glc}. Transcription from the promoter region of the *ptsH* and *ptsI* genes is stimulated 2.5- to 3-fold during growth on glucose compared with growth on pyruvate or glucose-6-phosphate as the sole carbon source. We showed that expression of a *ptsH-lacZ* or a *ptsH ptsI-lacZ* fusion in a strain deficient for EII^{Glc} (*ptsG*) was not modified during growth on glucose (5). This finding suggested an essential role of EII^{Glc} in this regulation. In a *ptsG* strain, TP2504, glucose is transported via the mannose-specific PTS enzymes. We thus wanted to test whether growth on mannose as a sole carbon source could stimulate expression of a *ptsH-lacZ* protein fusion (carried by plasmid pDIA3247). A twofold stimulation of expression of the fusion was observed in a *ptsG*$^+$ strain (Table 2) but only after a lag corresponding to the beginning of the exponential growth phase of the strain (data not shown). However, growth on mannose produced no stimulation in a *ptsG* strain (Table 2). Stock et al. (32) showed that mannose can be transported, although with less affinity, via EII^{Glc}. The observation that stimulation of the *ptsH-ptsI* expression was dependent on the presence of a *ptsG*$^+$ allele suggested that transport of mannose through EII^{Glc} is capable of generating the stimulatory signal. This result, taken together with the observation (5) that positive regulation can also be generated during growth in the presence of a nonmetabolized analog of glucose (methyl-α-glucoside, which is transported via EII^{Glc}-EIII^{Glc}), shows that translocation through EII^{Glc} rather than accumulation of a product of glucose transport or metabolism causes stimulation of expression of the *pts* operon.

Overproduction of EII^{Glc} causes stimulation of the *pts*

TABLE 2. Effect of mannose (strains TP2110 and TP2504) and of cAMP (strains TP2006 and TP2512) on β-galactosidase rate of synthesis of the *ptsH-lacZ* protein fusion carried by plasmid pDIA3247

Strain	Relevant genotype	β-Galactosidase synthesis (U/mg)[a]			
		Pyruvate	Mannose	Glycerol	Glycerol + cAMP
TP2110	*pts*$^+$ *cya*$^+$	5,600 (±400)	13,000 (±800)		
TP2504	*ptsG*	5,000 (±260)	3,200 (±250)		
TP2006	Δ*cya glp*$^+$[b]			2,400 (±150)	5,440 (±100)
TP2512	Δ*cya ptsG glp*$^+$[b]			2,100 (±100)	4,300 (±260)

[a] Rates of synthesis ($\Delta Z/\Delta B$ values) are expressed in Pardee units (17) per milligram (dry weight) of bacteria. Each number in parentheses corresponds to the 95% confidence limit of the corresponding $\Delta Z/\Delta B$ value.

[b] Able to grow on glycerol because it carries the *glp8306* mutation, which renders growth on glycerol independent of cAMP.

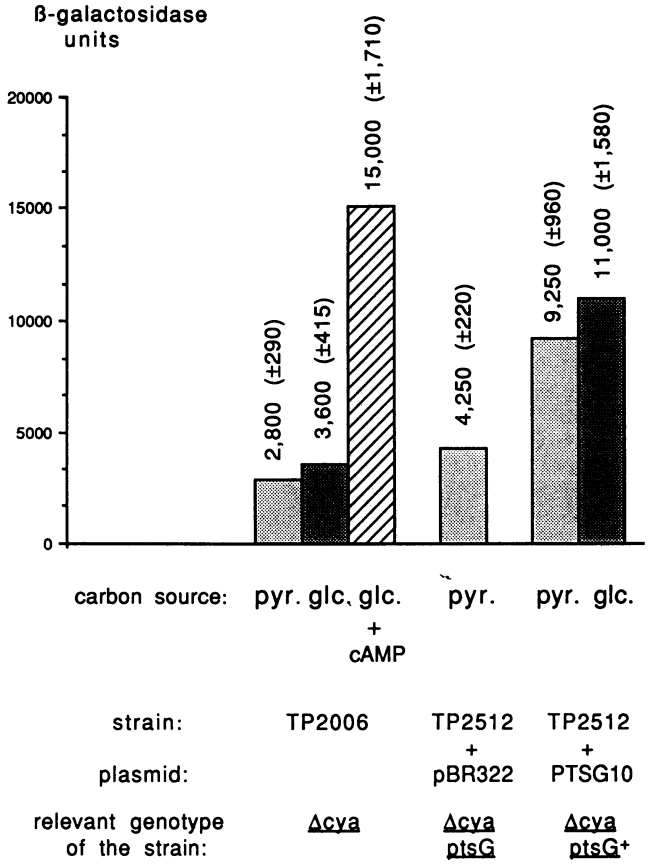

FIG. 2. β-Galactosidase rates of synthesis ($\Delta Z/\Delta B$ values expressed in Pardee units [17] per milligram [dry weight] of bacteria) of the following strains carrying plasmid pDIA3247: TP2006, TP2512 harboring the vector plasmid pBR322, and TP2512 containing a derivative of pBR322 carrying the *ptsG* gene, plasmid PTSG10. The precise $\Delta Z/\Delta B$ value is indicated above each bar. Each number in parentheses corresponds to the 95% confidence limit of the corresponding $\Delta Z/\Delta B$ value. Carbon sources: pyr., pyruvate; glc., glucose.

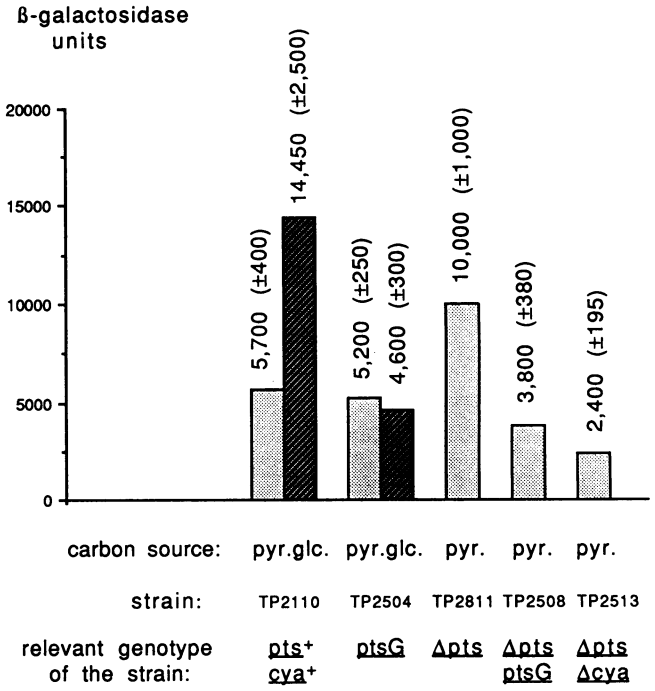

FIG. 3. β-Galactosidase rates of synthesis ($\Delta Z/\Delta B$ values expressed in Pardee units [17] per milligram [dry weight] of bacteria) of different strains carrying plasmid pDIA3260. The precise $\Delta Z/\Delta B$ value is indicated above each bar. Each number in parentheses corresponds to the 95% confidence limit of the corresponding $\Delta Z/\Delta B$ value. Carbon sources: pyr., for pyruvate; glc., glucose.

operon in the absence of CAP-cAMP. To determine whether expression of the *pts* operon was still regulated by growth on glucose in a strain with no cAMP (a Δ*cya* strain, deficient in adenylate cyclase), the following experiments were performed. We first measured expression of the *ptsH-lacZ* fusion (pDIA3247) in a Δ*cya* strain (TP2006) grown in a medium containing glucose compared with growth in a medium containing pyruvate (Fig. 2). We observed that glucose did not stimulate expression of the fusion alone, but that addition of cAMP to the glucose medium stimulated expression of the fusion fivefold compared with expression in a medium containing pyruvate (Fig. 2). This result could indicate that regulation during growth on glucose is indeed CAP-cAMP dependent. However, this lack of stimulation can also be explained by the earlier observations of Rephaeli and Saier (21) indicating that the *ptsG* gene encoding EII^{Glc} is under the positive control of CAP-cAMP.

To determine whether stimulation by glucose could occur independently of CAP-cAMP, we needed an alternative approach to synthesize EII^{Glc} in the absence of cAMP. We therefore used a high-copy-number plasmid (derived from pBR322) carrying the *ptsG* gene: plasmid PTSG10 (9). The Δ*cya ptsG* strain TP2512, carrying plasmid PTSG10, was transformed with the compatible *ptsH-lacZ* fusion of plasmid pDIA3247 (see Materials and Methods). During growth on glucose, expression of the fusion in this Δ*cya* strain in the presence of excess of EII^{Glc} (expressed from plasmid PTSG10) was stimulated approximately threefold (Fig. 2). In fact, during growth on pyruvate, a twofold stimulation of the fusion was also observed (Fig. 2). This interesting finding will be discussed below. These results thus show that regulation mediated by EII^{Glc} does not require CAP-cAMP.

Transcriptional activation by CAP-cAMP is independent of EII^{Glc}. Since expression of the *ptsG* gene is itself under the positive control of CAP-cAMP, we wondered whether the observed activation of the *ptsH-ptsI* promoter by CAP-cAMP could be an indirect consequence of an increase of the EII^{Glc} level in the presence of CAP-cAMP. The effect of cAMP on the *ptsH-lacZ* fusion was compared in two strains, TP2006 (Δ*cya*) and the double mutant TP2512 (Δ*cya ptsG*). Expression of the fusion was stimulated twofold by CAP-cAMP in both strains (Table 2). Activation by CAP-cAMP of the *pts* operon is thus not an indirect effect mediated by EII^{Glc}.

Stimulation of expression of the *pts* operon is dependent on the phosphorylation state of EII^{Glc}. Expression of the *ptsH-lacZ* fusion, carried on plasmid pDIA3260, was measured in strain TP2811 carrying a well-defined deletion of the whole *pts* operon Δ(*ptsH-ptsI-crr*) called Δ*pts* (14). During growth on pyruvate, expression of the fusion was significantly higher in the Δ*pts* strain TP2811 than in the isogenic *pts*$^+$ strain TP2110 (Fig. 3). In strain TP2508, carrying a *ptsG* mutation in addition to the deletion of the *pts* operon, the expression level of the fusion was lower than in the Δ*pts ptsG*$^+$ strain TP2811 (Fig. 3). In fact, expression of the

TABLE 3. Effect of glucose on β-galactosidase rate of synthesis of *ptsH-lacZ* protein fusion carried by plasmid pDIA3260

Strain	Relevant genotype	β-Galactosidase synthesis (U/mg)[a]	
		Pyruvate	Glucose
TP2110	*cya*$^+$ *pts*$^+$	5,700 (±400)	14,450 (±2,500)
TP2505	*crr*	3,700 (±360)	13,000 (±550)
TP2862	Δ*crr*	4,800 (±350)	13,700 (±1,480)
TP2509	*crr nagE*	4,300 (±920)	12,300 (±1,020)
TP2514	*crr nagE* Δ*bgl*	4,400 (±430)	14,000 (±1,160)

[a] Rates of synthesis (Δ*Z*/Δ*B* values) are expressed in Pardee units (17) per milligram (dry weight) of bacteria. Each number in parentheses corresponds to the 95% confidence limit of the corresponding Δ*Z*/Δ*B* value.

ptsH-lacZ fusion during growth on pyruvate was 2.5 times higher in strain TP2811 (Δ*pts*) than in strain TP2508 (Δ*pts ptsG*). This result shows that the presence of a functional (wild-type) *ptsG* gene in a Δ*pts* background results in a 2.5-fold enhancement of expression of the fusion. The same results were obtained with slightly different plasmids (data not shown). In contrast, expression of the fusion in a strain carrying the wild-type *pts* operon, grown on pyruvate, was not altered by the *ptsG* mutation (compare TP2110 [*pts*$^+$ *ptsG*$^+$] with TP2504 [*pts*$^+$ *ptsG*] in Fig. 3).

Expression of the *ptsH-lacZ* fusion was thus enhanced in a Δ*pts* strain in the absence of the inducer (glucose) but only in a *ptsG*$^+$ context. This result could be interpreted as suggesting that in strain TP2811, EII^{Glc} is in a state analogous to that during transport of glucose. In the Δ*pts* strain, there is no PEP-dependent phosphate transfer to the sugar-specific PTS proteins and, in particular, EII^{Glc} is unphosphorylated. This implies that the phosphorylation state of EII^{Glc} is a central element of the regulatory signal generated during transport, i.e., that stimulation of the *pts* operon is generated by an increase in the level of unphosphorylated EII^{Glc}. If expression of the *pts* operon was dependent only on the EII^{Glc} phosphorylation state, one would expect expression of the fusion in strain TP2811 (Δ*pts*) grown on pyruvate to be as elevated as in a wild-type strain grown on glucose (compare strains TP2811 and TP2110 in Fig. 3). The fact that it is only 70% of the value is almost certainly due to the reduction of the cAMP level in the Δ*pts* strain as measured previously by Lévy et al. (14). This interpretation is supported by the observation that the introduction of a Δ*cya* mutation (strain TP2513 [Δ*pts* Δ*cya*] in Fig. 3) reduced the expression of the fusion to a very low value.

Positive regulation by glucose is not mediated by EIII^{Glc}. It has been shown that phosphorylation of EII^{Glc} is dependent on active EIII^{Glc} (2, 18). Therefore, we tested whether EIII^{Glc} (encoded by the *crr* gene) could be the regulatory protein acting as a mediator between EII^{Glc} and the regulatory region of the *pts* operon. Expression of the *ptsH-lacZ* fusion (plasmid pDIA3260) in a strain carrying a *crr* mutation (TP2505) or a well-defined deletion of the *crr* gene (TP2862; 14) was tested. Stimulation of expression of the fusion was not affected by a *crr* mutation (Table 3). Surprisingly, expression of the fusion in strain TP2505 or TP2862 during growth on pyruvate was not as elevated as it was in the Δ(*ptsH-ptsI-crr*) strain TP2811 (Table 3 and Fig. 3). As it has been shown by Vogler et al. (34) that the C terminus of EII^{Nag} (encoded by *nagE*) or of EII^{Bgl} (encoded by *bglF*) could in certain circumstances replace the functions of a defective EIII^{Glc}, we tested the effect of these additional mutations on expression of the *ptsH-lacZ* fusion. Neither in strain TP2509 (*crr nagE*) nor in strain TP2514 (*crr nagE* Δ*bgl*) was the level of expression of the fusion significantly changed (Table 3).

DISCUSSION

The PTS provides a very efficient way of transporting carbohydrates into the bacterial cell. This system also exerts a regulatory effect on uptake and metabolism of a great number of non-PTS carbohydrates (27). This regulation can be considered in two categories: first, the PTS regulates the uptake of certain non-PTS carbohydrates; and second, it modulates the intracellular cAMP level, thereby regulating the expression of the operons sensitive to catabolite repression. In fact, the crucial variable for both types of regulation is the phosphorylation level of EIII^{Glc}, which is itself dependent on the availability of the two common PTS phosphoproteins, EI and HPr. Any change in *ptsH-ptsI* expression should thus have two consequences, a direct effect on the uptake of the PTS carbohydrates and an effect on the PTS-mediated regulation resulting from a change in the phosphorylation level of EIII^{Glc}. We were thus interested in determining the effectors modulating expression of *ptsH-ptsI* and how the modulations were brought about at the molecular level. We have been studying two factors acting on *ptsH-ptsI* expression, extracellular glucose and intracellular CAP-cAMP. Positive regulation by CAP-cAMP is a well-documented phenomenon, and the DNA sequence located upstream from the *ptsH* gene (present on plasmids pDIA3247 and pDIA3260) contains two sequences showing homology with the consensus of CAP binding sites (5). The principal aim of this study was to understand how the cell could sense the presence of glucose in the external medium.

We first wanted to know whether the two control circuits, via glucose and CAP-cAMP, were independent of each other. We show that EII^{Glc} has an essential role in the glucose-mediated regulation but that CAP-cAMP is able to activate independently of EII^{Glc}. In addition, we show that EII^{Glc} can cause activation in a Δ*cya* strain. The question of whether the two regulatory effects are additive is difficult to answer. Indeed, we observed, as described by Rephaeli and Saier (21), that expression of *ptsG* is strongly dependent on CAP-cAMP and this dependence causes the two regulations to be interrelated. It should be noted that there is an apparent contradiction between the CAP-cAMP-mediated activation of the *pts* operon and its stimulation during growth on glucose, which is a carbon source known to cause a dramatic decrease in the intracellular concentration of cAMP (33). Nevertheless, a tentative explanation can be given, since the existence of these two control circuits implies that synthesis of HPr and EI can be stimulated to provide sufficient phosphate transfer potential during growth on glucose as well as during growth on PTS substrates that do not cause a significant decrease in the intracellular concentration of cAMP, such as fructose or mannose.

The results presented here indicate that glucose acts on expression of the *pts* operon like an environmental signal, through a mechanism of signal transduction. We show that it is not just the presence of glucose in the medium but rather its translocation through the glucose-specific permease EII^{Glc} that is necessary for regulation. Stimulation of *ptsH-ptsI* expression in the presence of another PTS substrate, mannose, is dependent on an active EII^{Glc}, suggesting that this regulation is a consequence of mannose transport by EII^{Glc}. This stimulation, however, occurred after a lag corresponding to the beginning of the exponential growth

phase. This finding could be accounted for by the fact that since EII^{Glc} has low affinity for mannose (32), transport through this permease becomes significant only after all the EII^{Man} enzymes have been saturated by mannose. In addition, this finding suggests that at least in this genetic background, transport through EII^{Man} does not significantly stimulate the *pts* operon expression.

Such a regulatory mechanism dependent on the uptake of glucose rather than on the accumulation of a product of its transport or metabolism is probably designed to avoid a constant activation in the presence of substantial levels of glucose-6-phosphate or glucose produced inside the cell by the uptake of other carbon sources.

We wondered what the molecular signal generated inside the cell during EII^{Glc}-dependent glucose uptake could be. Saier (26) proposed that dephosphorylation of EII^{Glc} might generate such a signal. Our results strongly suggest that this is indeed the case. We showed that in a Δ(*ptsH-ptsI-crr*) strain unable to phosphorylate the PTS proteins, expression of the *ptsH-lacZ* fusion was stimulated in the absence of glucose (the inducer) but only in the presence of an active EII^{Glc}. Consistent with this finding is the observation that when an excess of EII^{Glc} is present in the cell due to the presence of a multicopy plasmid carrying the *ptsG* gene (PTSG10), expression of the *pts* operon is stimulated in a noninducing medium. Indeed, it is likely that in such a strain there will be a higher level of nonphosphorylated EII^{Glc} than in a strain without plasmid PTSG10. The presence of a multicopy *ptsG* gene should increase the level of nonphosphorylated EII^{Glc} either because of the limiting capacity of EI, HPr, and $EIII^{Glc}$ or, if the phosphorylation is not limiting, because the absolute values of both P-EII^{Glc} and EII^{Glc} are high.

We conclude from these results that either EII^{Glc} directly or, more likely, a regulatory protein which interacts with EII^{Glc} is capable of regulating transcription of *ptsH-ptsI* in a way dependent on the phosphorylation state of EII^{Glc}. Although the properties of $EIII^{Glc}$ made it an excellent candidate for such a regulatory protein, our results clearly indicated that $EIII^{Glc}$ was not necessary for the glucose-mediated regulation of the *pts* operon. Vogler et al. (34) have shown that the $EIII^{Glc}$-like domains of EII^{Nag} and EII^{Bgl} can replace $EIII^{Glc}$ in $EIII^{Glc}$-dependent transport and phosphorylation. However, regulation of the *pts* operon by glucose remained unaltered in the *crr nagE* and *crr nagE Δbgl* mutants. In contrast to what was observed in the Δ*pts* strain, expression of the *pts* operon is not stimulated in the Δ*crr*, *crr nagE*, and *crr nagE Δbgl* strains grown in the absence of glucose. Since our results show that regulation of the *pts* operon depends on the phosphorylation state of EII^{Glc}, we are forced to conclude that in a *crr nagE Δbgl* strain, there is still sufficient phosphorylation of EII^{Glc} to prevent stimulation of *ptsH-ptsI* expression. On the basis of the observation that a *crr nagE* strain of *Salmonella typhimurium* still shows EII^{Glc}-dependent transport activity, Vogler et al. (34) also proposed the existence of other EIIs, not yet identified, which might contain stretches of amino acids homologous to those of $EIII^{Glc}$ and therefore might be able to substitute for $EIII^{Glc}$ in transport and phosphorylation activities. It can even be proposed that another EIII domain, less homologous to $EIII^{Glc}$, could be able to phosphorylate EII^{Glc} without allowing efficient transport of glucose through this permease. Finally, one cannot completely exclude the possibility that in this *crr nagE Δbgl* strain, EII^{Glc} is phosphorylated directly by P-HPr or at the expense of a sugar-phosphate, for instance, glucose-6-phosphate.

Expression dependent on the phosphorylation of a regulatory protein has been described for the *bgl* operon, which encodes the PTS genes specific of the uptake of the β-glucosides. Expression of these genes is dependent on the phosphorylation state of EII^{Bgl}. However, regulation of the *bgl* operon is different from that of the *pts* operon since it is positively regulated by an antitermination protein which is itself negatively controlled, through phosphorylation, by EII^{Bgl} (1). Regulation by an extracellular inducer has already been described in the case of two transport systems of carbon sources: the sugar-phosphate transport system of *E. coli* (the Uhp system; 35) and the phosphoglycerate transport system of *S. typhimurium* (the Pgt system; 36). The exogenous induction of the *uhp* genes is mediated by a mechanism different from that described here, since transport of the inducer (glucose-6-phosphate) is not needed for induction. These two signal transduction systems (Uhp and Pgt) are part of the large family of the so-called two-component systems which are controlled by members of two homologous families of proteins, the sensor proteins and the regulator proteins (31). It is interesting that most of these systems respond to environmental signals and that in a number of cases it has clearly been shown that the sensor protein is a histidine protein-kinase. In these systems, phosphorylation on a His residue has been shown to be the signal transmitted from a sensor protein to a regulator protein acting on gene expression (the Uhp, Pgt, Che, and Omp systems; see reference 31). It has been shown that many of these histidine kinases are membrane-associated proteins. It should be recalled that the phosphoryl groups of almost all of the PTS proteins are carried by His residues, although in this case it is the result of group translocation rather than an ATP-requiring kinase. However, for the two-component systems as for the regulation of the *pts* operon, phosphorylation on His residues of response regulators is an essential feature of the signal transduction mechanism.

The striking analogy between these two-component systems and the glucose-mediated regulation of the *pts* operon is strengthened by the following observation. Kofoid and Parkinson (11, 11a) showed that a stretch of 209 residues at the C terminus of EII^{Glc} exhibits a sequence similarity of 39% with the transmitter consensus motif of sensor proteins that they defined. Consistent with the properties expected for a transmitter module, the C-terminal end of EII^{Glc} comprising about 90 residues has hydrophilic properties, and biochemical experiments suggest that this region might be exposed at the cytoplasmic face of the membrane (8). These observations argue in favor of the notion that the membrane-bound EII^{Glc} might be a sensor protein resembling those of the two-component systems. Before this hypothesis can be substantiated, it is necessary to identify the second component of the system, the effector molecule. The experiments described here seem to have eliminated the possibility that $EIII^{Glc}$ plays this role. We are now performing an extensive analysis of the DNA region located upstream from the *ptsH* gene. This study should lead to identification of the regulatory sites and subsequently of the proteins involved in regulation. This will be helpful for an understanding of the mechanisms involved in regulation of expression of the *pts* operon.

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