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cAMP receptor protein–cAMP plays a crucial role in glucose–lactose diauxie by activating the major glucose transporter gene in *Escherichia coli*

KEIKO KIMATA*, HIDEYUKI TAKAHASHI*, TOSHIFUMI INADA*, PIETER POSTMA†, AND HIROJI AIBA*‡

*Department of Molecular Biology, School of Science, Nagoya University, Chikusa, Nagoya 464-01, Japan; and †E. C. Slater Institute, BioCentrum, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands

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ABSTRACT **The inhibition of β-galactosidase expression in a medium containing both glucose and lactose is a typical example of the glucose effect in *Escherichia coli*. We studied the glucose effect in the *lacL8UV5* promoter mutant, which is independent of cAMP and cAMP receptor protein (CRP). A strong inhibition of β-galactosidase expression by glucose and a diauxic growth were observed when the *lacL8UV5* cells were grown on a glucose–lactose medium. The addition of isopropyl β-D-thiogalactoside to the culture medium eliminated the glucose effect. Disruption of the *crr* gene or overproduction of LacY also eliminated the glucose effect. These results are fully consistent with our previous finding that the glucose effect in wild-type cells growing in a glucose–lactose medium is not due to the reduction of CRP–cAMP levels but is due to the inducer exclusion. We found that the glucose effect in the *lacL8UV5* cells was no longer observed when either the *crp* or the *cya* gene was disrupted. Evidence suggested that CRP–cAMP may not enhance directly the *lac* repressor action *in vivo*. Northern blot analysis revealed that the mRNA for *ptsG,* a major glucose transporter gene, was markedly reduced in a Δ*crp* or Δ*cya* background. The constitutive expression of the *ptsG* gene by the introduction of a multicopy plasmid restored the glucose effect in Δ*cya* or Δ*crp* cells. We conclude that CRP–cAMP plays a crucial role in inducer exclusion, which is responsible for the glucose–lactose diauxie, by activating the expression of the *ptsG* gene.**

In enteric bacteria, the synthesis of many catabolic enzymes is inhibited by the presence of glucose in the growth medium. Multiple mechanisms are involved in this phenomenon, referred to as "glucose effect" or "glucose repression" (1–5). Although glucose signaling may occur via different pathways, glucose ultimately would affect the transcription of catabolic operons by modulating transcription factor(s). In the lactose operon of *Escherichia coli*, the final targets of glucose are the *lac* repressor and the positive regulator, the complex of cAMP receptor protein (CRP) and cAMP. First, glucose prevents the entry of inducer into the cell, resulting in an increase in the concentration of the inducer-free *lac* repressor. The mechanism of this process, called "inducer exclusion," is relatively well understood (3–5). The transport of glucose into the cell by the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) decreases the level of phosphorylation of enzyme IIA^{Glc}, one of the enzymes involved in glucose transport. The dephosphorylated enzyme IIA^{Glc} binds to and inactivates the *lac* permease, causing the inducer exclusion. Second, glucose lowers the level of CRP–cAMP by reducing the intracellular concentrations of both cAMP and CRP under certain conditions, for example, when added to cells growing on a poor carbon source such as glycerol or succinate (6, 7). Glucose is thought to reduce cAMP level by decreasing the phosphorylated form of enzyme IIA^{Glc}, which is proposed to be involved in the activation of adenylate cyclase (3–5). Glucose also is known to reduce the CRP level through the autoregulation of the *crp* gene (7–10).

When *E. coli* finds both glucose and lactose in the medium, it preferentially uses the glucose, and the use of lactose is prevented until the glucose is used up, causing a biphasic growth (diauxie)(11, 12). The glucose–lactose diauxie is a prototype of the glucose effect. Concerning the mechanisms that lead to the inhibition of the *lac* operon expression, it widely has been believed that glucose inhibits *lac* expression by reducing the level of cAMP and therefore by depriving the *lac* operon of a transcriptional activator (CRP–cAMP) necessary for its expression.

Recently, we challenged this famous "cAMP model" and found that the level of CRP–cAMP in lactose-grown cells was essentially the same as that in glucose-grown cells (13). We also showed that disruption of the *lacI* gene completely abolished the glucose effect. These and other data have led us to conclude that the reduction in the CRP–cAMP level cannot be responsible for the glucose effect in the glucose–lactose system and that glucose prevents the expression of the *lac* operon by enhancing *lac* repressor activity (13).

The above finding does not exclude the possibility that CRP–cAMP plays any other role(s) in the diauxie, however. It is known that CRP–cAMP is involved in the expression of several PTS proteins, including those required for glucose uptake and phosphorylation (3, 4). It is possible that, in this way, the activity of the *lac* repressor is affected by glucose. Alternatively, CRP–cAMP might be involved in the glucose effect by directly enhancing the *lac* repressor action through cooperative binding at the *lac* promoter (14, 15). To test these possibilities, we investigated the glucose effect in the *lacL8UV5* mutant in which the *lac* promoter is independent of CRP–cAMP (16). We found that both CRP and cAMP are required for the glucose effect. In addition, we showed that the expression of *ptsG,* a major glucose transporter gene, is under the control of CRP–cAMP. We conclude that CRP–cAMP plays a crucial role in the inducer exclusion, which is responsible for glucose–lactose diauxie, by activating the transcription of *ptsG* gene.

MATERIALS AND METHODS

Media and Growth Conditions. Cells were grown aerobically at 37°C in M9 medium (17) supplemented with 0.001%

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Abbreviations: CRP, cAMP receptor protein; PTS, phosphoenolpyruvate-dependent carbohydrate phosphotransferase system; IIA^{Glc}, glucose-specific IIA protein; $IICB^{Glc}$, glucose-specific IICB protein.

‡To whom reprint requests should be addressed. e-mail: i45346a@nucc.cc.nagoya-u.ac.jp.

thiamine or in Luria–Bertani medium (17). Antibiotics were used at the following concentrations: ampicillin (50 μg/ml), kanamycin (50 μg/ml), and tetracycline (15 μg/ml). Bacterial growth was monitored by determining the OD at 600 nm.

Bacterial Strains and Plasmids. The *E. coli* K-12 strains and plasmids used in this study are listed in Table 1. KK8 was constructed by P1 transduction using HT28 as a donor strain. KK15 and KK17 were constructed by P1 transduction using IT1409. KK20 and KK21 were constructed by P1 transduction using IT1168 and IT1199, respectively. Construction of HT28, IT1409, IT1168, and IT1199 will be described elsewhere. The 3.6-kb *Bam*HI–*Sal*I fragment, containing the *cya* gene without its 5′ portion, derived from pIT228 (19), was inserted into the corresponding sites of pSTV28 (Takara Shuzo, Kyoto) to construct pIT298. The *Bam*HI–*Xba*I fragment of pIT298 was cloned into the corresponding sites of pACYC184. Subsequently, the *Bam*HI fragment containing the 5′ portion of *cya* under the control of the *bla* promoter was prepared from pSE3 (19) and inserted into the *Bam*HI site of pIT298 to construct pIT302. The 4.8-kb *Hin*dIII–*Acc*I fragment carrying the *ptsH* and *ptsI* genes prepared from Y. Kohara's library (20) was cloned into pBR322 to construct pST51. The *Sal*I–*Sal*I DNA fragment containing the *ptsG* gene derived from Kohara's library was cloned into the *Sal*I site of pSTV28 to construct pIT499. The *Mlu*I site located 50 bp upstream of the *ptsG* start codon in pIT499 was changed to a *Hin*dIII site to construct pTH110. The 2.5-kb *Hin*dIII–*Eco*RI fragment of pTH110 carrying the entire structural gene for *ptsG* was cloned into the corresponding sites of pBR322 to construct pTH111. The 6-kb *Eco*RI–*Pst*I fragment containing the *lacYA* genes was cloned into the corresponding sites of pBR322 to construct pIT539, in which the *lacYA* genes are expressed from the *bla* promoter.

Northern Blot Analysis. Cells were grown on M9 media containing carbon sources, and total RNAs were extracted as described (21). The RNAs were resolved by agarose-gel electrophoresis in the presence of formamide and blotted onto Hybond-N^+ membrane (Amersham) as described (22). The DNA probes were labeled with [α-^{32}P] dCTP by random priming. The membranes were hybridized and washed, and the signals were visualized by autoradiography.

β-Galactosidase Assay. β-Galactosidase activity was determined with permeabilized cells by the method of Miller (17).

RESULTS

Glucose Effect in the *lacL8UV5* Strain. We first investigated the effect of glucose on the expression of β-galactosidase in strain PR166, which carries the *lacL8UV5* variant on a F′ plasmid in a *lacZYA* deletion background (16). The *L8* mutation is located in the CRP binding site in the *lac* promoter (23) and reduces the promoter activity by inhibiting CRP–cAMP binding (23) and/or by altering conformation of the CRP–DNA complex (24). The *UV5* mutation, originally isolated as a suppressor of the *L8* mutation (25), alters the −10 sequence such that it completely fits the consensus −10 sequence and enhances the promoter activity (23). Thus, the *lacL8UV5* promoter has little ability to respond to CRP–cAMP (16). In fact, the β-galactosidase activity in PR166 cells growing on lactose medium was essentially the same as that in isogenic Δ*crp* cells (see Figs. 1*A* and 2*A*). If a reduction in cAMP level caused the glucose–lactose diauxie, the glucose effect would be abolished in the *lacL8UV5* strain because the transcription of this promoter no longer requires CRP–cAMP. On the other hand, if the modulation of the *lac* repressor activity through the inducer exclusion was responsible for the glucose effect, one might expect that the *L8UV5* mutation would not affect the glucose effect. We observed a typical diauxie and a strong repression of β-galactosidase activity in the *lacL8UV5* mutant, as was the case in wild-type cells (Fig. 1*A*). In other words, the glucose effect was independent of the positive regulation of the *lac* operon by CRP–cAMP. The presence of isopropyl β-D-thiogalactoside in the growth medium completely eliminated the glucose effect (Fig. 1*B*). In addition, the disruption of the *crr* gene coding for IIA^{Glc} (Fig. 1*C*) or the overproduction of Lac permease (Fig. 1*D*) essentially eliminated the glucose effect. These results are fully consistent with our claim that the inducer exclusion, mediated by IIA^{Glc}, but not the reduction in cAMP levels is responsible for the glucose–lactose diauxie.

CRP–cAMP Is Required for the Glucose Effect in the *lacL8UV5* Strain. To examine whether CRP–cAMP plays any role(s) in the glucose effect, we disrupted the *cya* or *crp* gene in PR166. Interesting to note, the disruption of the *crp* gene completely eliminated the glucose effect (Fig. 2*A*). The diauxic growth and the strong repression of β-galactosidase activity by glucose in the *lacL8UV5* Δ*crp* cells were restored by the introduction of pHA7, carrying the *crp* gene (Fig. 2*B*). The disruption of the *cya* gene also eliminated the glucose effect in the *lacL8UV5* mutant, and the introduction of pIT302 carrying the *cya* gene restored the glucose effect (see Fig. 5*B*). These results clearly indicate that CRP–cAMP is required for the glucose effect.

Table 1. Bacterial strains and plasmids used in this study

Strain/plasmid	Relevant genotype and property	Source
Strain		
W3110	Wild-type	Laboratory stock
HT28	W3110 Δ*cya*::Kan	This study
IT1409	W3110 Δ*crp*::Tet	This study
IT1168	W3110 *ptsG*::Tn5	This study
IT1199	W3110 Δ*crr*::Kan	This study
PR158	*galK2 str*^{R} *sup*^{o} *lacZYA21 F′plac*$^+$ *lacZ*$^+$	ref. 16
PR166	*galK2 str*^{R} *sup*^{o} *lacZYA21 F′placL8UV5lacZ*$^+$	ref. 16
KK8	PR166 Δ*cya*::Kan	This study
KK15	PR158 Δ*crp*::Tet	This study
KK17	PR166 Δ*crp*::Tet	This study
KK20	PR166 *ptsG*::Tn5	This study
KK21	PR166 Δ*crr*::Kan	This study
Plasmid		
pHA7	Derivative of pBR322 containing the *crp* gene expressed from the *bla* promoter	ref. 18
pIT302	Derivative of pACYC184 containing the *cya* gene expressed from the *bla* promoter	This study
pTH111	Derivative of pBR322 containing the *ptsG* gene expressed from *bla* promoter	This study
pST51	Derivative of pBR322 containing the *ptsHI* genes expressed from the *pts* promoter	This study
pIT539	Derivative of pBR322 containing the *lacYA* genes expressed from the *bla* promoter	This study

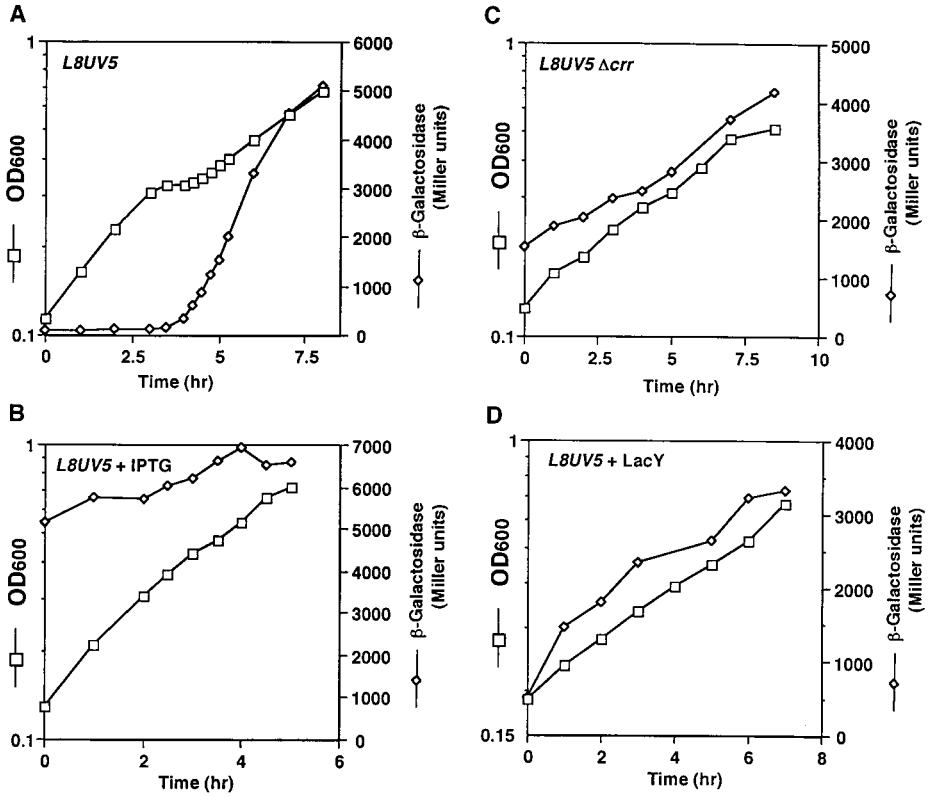

FIG. 1. Growth curve and β-galactosidase activity of *lacL8UV5* cells growing on a glucose–lactose medium**.** Cells were grown in M9 medium containing 0.04% glucose and 0.2% lactose. The following strains and addition were used: (*A*) PR166; (*B*) PR166 plus 0.5 mM isopropyl β-D-thiogalactoside; (*C*) KK21; and (*D*) PR166 harboring pIT539. At the indicated time, samples were removed to determine the OD (squares) and β-galactosidase activity (diamonds).

CRP–cAMP May Not Directly Enhance Repressor Action. How does CRP–cAMP participate in the glucose effect? One attractive hypothesis is that CRP–cAMP would directly enhance *lac* repressor binding to the operator. In fact, it was reported that the ternary complex of CRP–cAMP and *lac* repressor bound to their respective binding sites is more stable than would be expected based on the affinities of independently bound proteins *in vitro* (14, 15). The crystallographic structure of the *lac* repressor–DNA complex also suggested that CRP–cAMP functions synergistically with the *lac* repressor and participates in the formation of a repression loop (26). If repressor binding to the operator were enhanced by CRP–cAMP *in vivo*, one might expect the expression of β-galactosidase in *lacL8UV5*Δ*crp* cells to be higher than that in isogenic *crp*$^+$ cells. However, we found that this was not the case (Table 2). One could argue that the failure of CRP to affect the binding of repressor in *L8UV5* is due to the mutation in the CRP binding site. Therefore, we determined the β-galactosidase activity in strains carrying the wild-type lactose operon. The β-galactosidase activity in the *crp*$^+$ cells was rather higher than that in the isogenic Δ*crp* cells (Table 2). These data seem to be in conflict with the view that the presence of CRP–cAMP directly enhances *lac* repressor action *in vivo.*

The Reduced Expression of *ptsHI* Is Not Responsible for the Failure of the Glucose Effect in Δ*cya* or Δ*crp* Cells. Another possible role of CRP–cAMP in the glucose effect is to enhance indirectly repressor action by modulating the PTS that is responsible for the inducer exclusion. Indeed, it is known that the expression of many PTS proteins is regulated by CRP–cAMP (3, 4). Several PTS proteins are involved in the uptake and phosphorylation of glucose. The major glucose transporter of *E. coli* consists of two components, cytoplasmic IIA^{Glc} encoded by the *crr* gene and transmembrane $IICB^{Glc}$ encoded by the *ptsG* gene. In addition, the *ptsH* and *ptsI* genes for the general PTS proteins HPr and enzyme I, respectively, are required for the uptake and phosphorylation of glucose (3, 4). It has been reported that the transcription of the *pts* operon containing the *ptsH*, *ptsI*, and *crr* genes is activated severalfold by CRP–cAMP although the *crr* gene is predominantly transcribed from another constitutive promoter located within the 3′ end of *ptsI* (27). We examined the effect of CRP–cAMP on the expression of *ptsHI* by Northern blot analysis (Figs. 3*A* and 4*A*). The expression of *ptsHI* was reduced moderately by the disruption of the *crp* or *cya* gene as expected (Fig. 4*A*). To examine the role of expression of the *ptsHI* genes in the glucose effect, we introduced a multicopy plasmid pST51, carrying the *ptsHI* genes, into the Δ*cya* or Δ*crp* strain. The introduction of pST51 overproduced the *ptsHI* RNA (Fig. 4*A*) but did not restore the glucose effect in *lacL8UV5* cells that contained Δ*cya* or Δ*crp* (Fig. 4*B*). The results indicate that the reduced expression of *ptsHI* is not responsible for the failure of the glucose effect in Δ*cya* or Δ*crp* cells.

The Expression of *ptsG* Is Strongly Dependent on CRP–cAMP. Concerning the regulation of the *ptsG* gene, it was reported that the activity of the glucose-specific enzyme II complex (IIA^{Glc} + $IICB^{Glc}$) was low in *crp* or *cya* mutants compared with the isogenic wild-type strain (28). This suggests that the expression of the *ptsG* gene is positively regulated by CRP–cAMP. However, no data are available on the transcriptional regulation of the *ptsG* gene by CRP–cAMP. We performed a Northern blotting experiment to investigate the regulation of *ptsG* expression by CRP–cAMP (Figs. 3*B* and 5*A*). When a DNA probe corresponding to a part of the structural gene of the *ptsG* (29) was used, a major mRNA specific for the *ptsG* was detected in a *crp*$^+$ *cya*$^+$ background (Fig. 5*A*). Interesting to note, little *ptsG* mRNA was visualized in a Δ*crp* or Δ*cya* background. The presence of pHA7 or

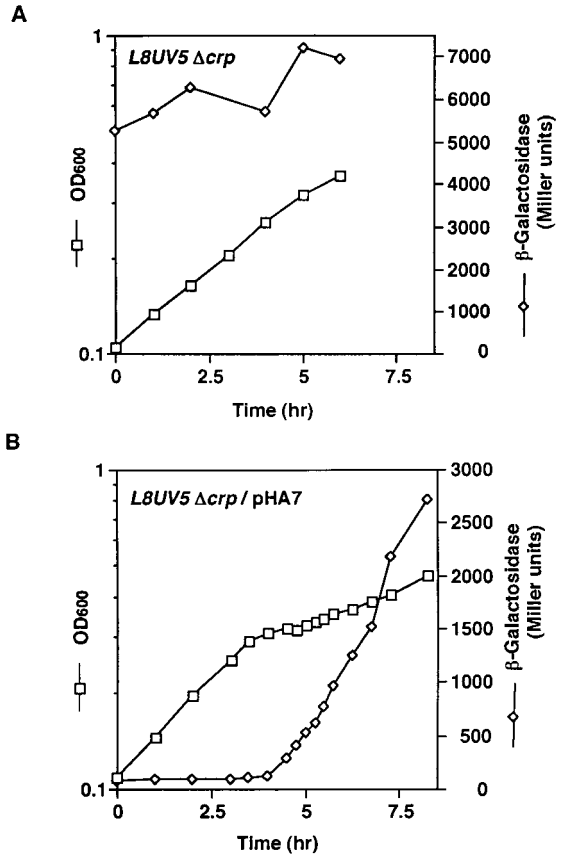

FIG. 2. Glucose effect in *lacL8UV5* Δ*crp* cells. KK17 (*A*) or KK17 harboring pHA7 (*B*) were grown in M9 medium containing 0.04% glucose and 0.2% lactose. At the indicated time, samples were removed to determine the OD (squares) and β-galactosidase activity (diamonds).

pIT302 restored the expression of *ptsG* mRNA in Δ*crp* and Δ*cya* cells, respectively. These results strongly suggest that the transcription of *ptsG* is under the control of CRP–cAMP.

The Reduced Expression of ptsG Is Responsible for the Failure of the Glucose Effect in Δ*cya* or Δ*crp* Cells. We reasoned that the failure of the glucose effect in Δ*crp* or Δ*cya* cells may be due to the reduction of the expression of *ptsG*. In fact, the disruption of the *ptsG* gene in crp^+ cya^+ background eliminated the glucose effect (Fig. 5*B*). To verify this conclusion, we introduced a multicopy plasmid pTH111, in which the *ptsG* is expressed constitutively under the *bla* promoter, into Δ*crp* or Δ*cya* cells. Northern blot analysis revealed that the *ptsG* mRNA levels in Δ*crp* or Δ*cya* cells carrying pTH111 were expressed highly(Fig. 5*A*). The size of *ptsg* mRNA derived from pTH111 is slightly shorter than that of the native *ptsg* mRNA

Table 2. Effect of CRP on the *lac* expression in the absence of inducer

lac promoter	*crp*	Strain	β-Galactosidase activity
L8UV5	+	PR166	55
	−	KK17	37
Wild-type	+	PR158	26
	−	KK15	6

Cells were grown at 37°C in Luria–Bertani medium to OD_{600} = 0.6. β-Galactosidase activity was determined and expressed in Miller units (17). Each value is the average of three experiments.

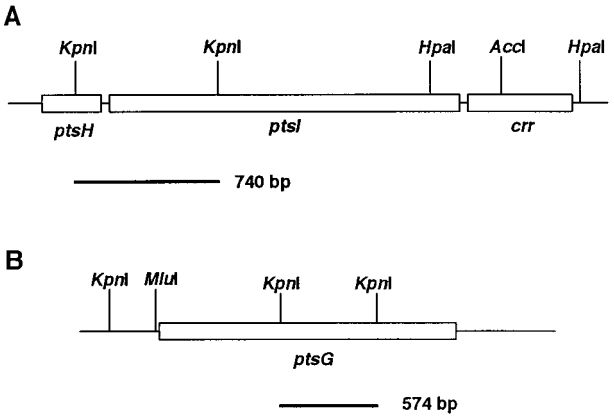

FIG. 3. DNA probes for Northern blotting experiment. (*A*) The restriction map of the DNA region around the *ptsH, ptsI*, and *crr* genes. (*B*) The restriction map of the DNA region around the *ptsG* gene. The data were taken from refs. 27 and 29, respectively. The open boxes indicate the coding region. Black lines beneath the map represent the DNA probes used for Northern blotting.

due to the use of the *bla* promoter. Then, we investigated the effect of glucose on the β-galactosidase expression in Δ*crp* or Δ*cya* cells carrying the plasmid in two conditions. First, the cells were grown in a M9 medium containing 0.5% glucose and 0.5% lactose, and the β-galactosidase activities were determined at OD_{600} = 0.6. As shown in Fig. 5*B*, the introduction of the *ptsG* plasmid completely restored the glucose effect in Δ*crp* or Δ*cya* cells. Second, the cells were grown in a M9 medium containing 0.04% glucose and 0.2% lactose, and the β-galactosidase expression was monitored during cell growth

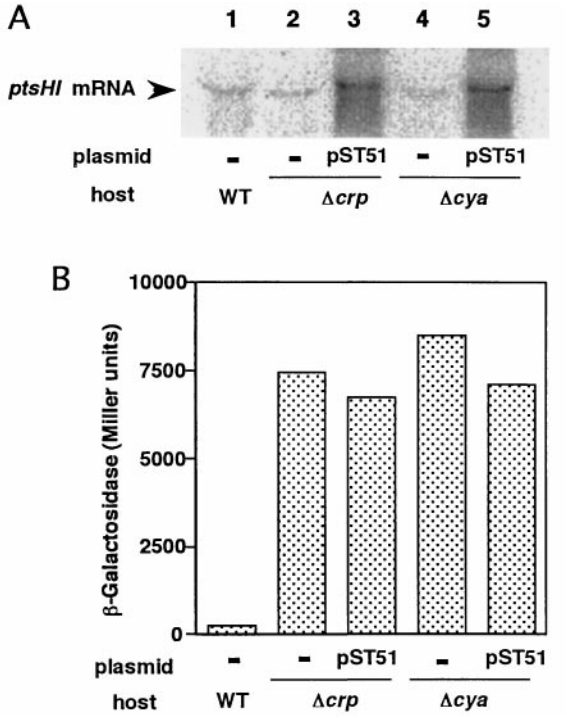

FIG. 4. Glucose effect and the expression of *ptsHI* genes in *lacL8UV5* cells. (*A*) Northern blot analysis of the *ptsHI* mRNA. Cells were grown in M9 medium containing 0.5% glucose and 0.5% lactose. Total RNAs, 50 μg (lanes 1, 2, and 4) or 5 μg (lanes 3 and 5), isolated from PR166 (lane 1), KK17 (lane 2), KK17 harboring pST51 (lane 3), KK8 (lane 4), and KK8 harboring pST51 (lane 5) were subjected to Northern blot analysis. (*B*) Cells were grown in M9 medium containing 0.5% glucose and 0.5% lactose. β-Galactosidase activity was determined at OD_{600} = 0.6. Each value is the average of three experiments.

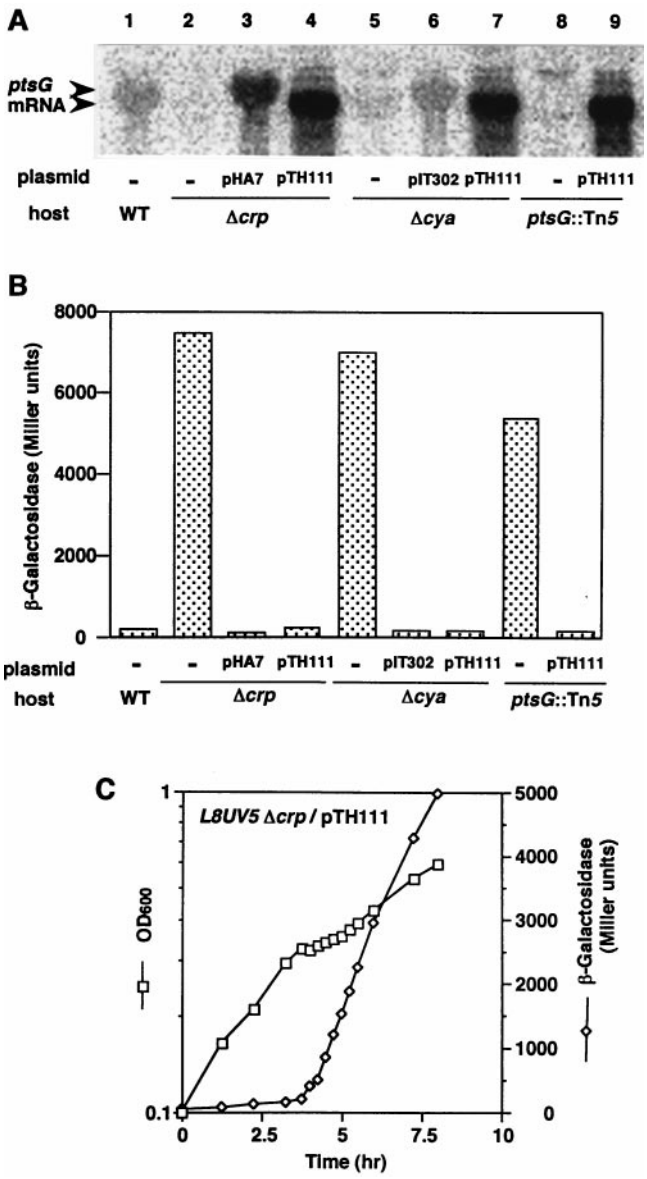

FIG. 5. Glucose effect and the expression of *pts*G gene in *lacL8UV5* cells. (*A*) Northern blot analysis of the *ptsG* mRNA. Cells were grown in M9 medium containing 0.5% glucose and 0.5% lactose. Total RNAs, 50 μg (lanes 1, 2, 3, 5, 6 and 8) or 5 μg (lanes 4, 7 and 9), isolated from PR166 (lane 1), KK17 (lane 2), KK17 harboring pHA7 (lane 3), KK17 harboring pTH111 (lane 4), KK8 (lane 5), KK8 harboring pIT302 (lane 6), KK8 harboring pTH111 (lane 7), KK20 (lane 8), and KK20 harboring pTH111(lane 8) were subjected to Northern blot analysis. (*B*) Cells were grown in M9 medium containing 0.5% glucose and 0.5% lactose. β-Galactosidase activity was determined at OD_{600} of 0.6. Each value is the average of three experiments. (*C*) KK17 cells harboring pTH111 were grown in M9 medium containing 0.04% glucose and 0.2% lactose. At the indicated time, samples were removed to determine the OD (squares) and β-galactosidase activity (diamonds).

(Fig. 5*C*). Diauxic growth and strong repression of β-galactosidase activity by glucose were observed. These results clearly indicate that the reduced expression of *ptsG* is responsible for the failure of the glucose effect in the absence of CRP–cAMP.

DISCUSSION

Transcription of the *E. coli lac* operon in a lactose-containing medium is strongly repressed by the presence of glucose, resulting in a diauxic growth curve. Because the *lac* operon is under both negative and positive transcriptional control by the *lac* repressor and CRP–cAMP, respectively (23, 30), glucose could inhibit *lac* transcription by increasing the level of unliganded repressor and/or by decreasing the level of CRP–cAMP in the cell. Previously, we presented evidence that the glucose effect in a glucose–lactose system is not due to a reduction in CRP–cAMP, as generally believed, but is due to the activation of *lac* repressor through inducer exclusion (13). In this paper, we addressed the question whether CRP–cAMP affects the repressor action and, if so, how it modulates the repressor activity.

First, we showed that glucose strongly inhibited *lac* expression, resulting in a typical diauxie in the *lacL8UV5* strain, as was the case in a strain with the wild-type *lac* promoter. We also showed that either the disruption of the *crr* gene or the overproduction of LacY eliminated the glucose effect. These data are completely consistent with our previous conclusion that the glucose–lactose diauxie is not mediated by a decreased concentration of CRP–cAMP (13). Second, we found that the glucose effect in the *lacL8UV5* strain was no longer observed in a Δ*crp* or Δ*cya* background. Thus, CRP–cAMP was shown to be required for the glucose effect. Third, we found that the level of the *ptsG* mRNA is reduced markedly in Δ*crp* or Δ*cya* cells and that the introduction of a *ptsG* plasmid restored the glucose effect.

Based on these results, we concluded that the reduction in *ptsG* mRNA level is likely to be responsible for the failure of the glucose effect in Δ*crp* or Δ*cya* cells and that an important role of CRP–cAMP in the glucose effect is to support inducer exclusion by activating the *ptsG* expression. A model explaining the role of CRP–cAMP in the inducer exclusion by glucose is presented in Fig. 6. The importance of the $IICB^{Glc}$ level in inducer exclusion has been shown previously (31, 32), namely, there is no inducer exclusion elicited by methyl α-glucoside below a certain level of $IICB^{Glc}$. It is most likely that the *ptsG* gene belongs to the CRP–regulon. In fact, preliminary experiments indicate that the transcription of the *ptsG* gene is stimulated markedly by CRP–cAMP *in vitro* (unpublished data). It should be noted that cells lacking $IICB^{Glc}$ can still take up glucose, although less efficiently, through several other PTS proteins such as II^{Man} complexes (4). This means that glucose

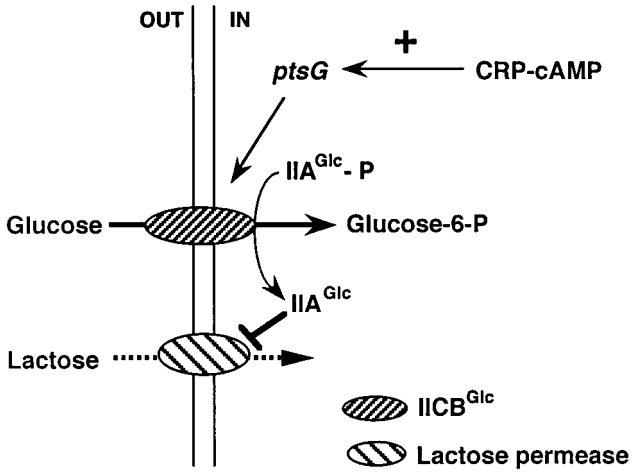

FIG. 6. A model explaining the role of CRP–cAMP in the glucose effect in the glucose–lactose system. The transport and phosphorylation of glucose by glucose PTS (IIA^{Glc} + $IICB^{Glc}$) increase the dephosphorylated IIA^{Glc} that prevents the uptake of lactose by inhibiting the *lac* permease activity. An important role of CRP–cAMP in the glucose effect is to support inducer exclusion by activating the *ptsG* transcription. The reduction in *ptsG* transcription is responsible for the failure of the glucose effect in the absence of CRP–cAMP.

must be incorporated into cells through the $IIA^{Glc}/IICB^{Glc}$ system to exhibit "the glucose effect."

In addition to the regulation by CRP–cAMP, expression of the *ptsG* gene appears to be induced by glucose (29, 33). The transcription of the *ptsHI* operon also is known to be stimulated by both glucose and CRP–cAMP (27, 34). Because the levels of CRP and cAMP are reduced by glucose, the expression of the glucose PTS should be regulated not only by CRP–cAMP but also by other factors that mediate the effect of glucose. The mechanism by which glucose stimulates the expression of *ptsG* and *ptsHI* genes is largely unknown.

We also examined a possibility that CRP–cAMP might be involved in the glucose effect by directly enhancing *lac* repressor binding to its operator. Although our experiments suggest that CRP–cAMP may not directly enhance the repressor action *in vivo*, at least under our experimental conditions, we cannot rule out the possibility that cooperative interaction between CRP–cAMP and the *lac* repressor plays some other role in the regulation of the *lac* operon. For example, it has been proposed that the *lac* repressor–CRP cooperativity could act to sequester RNA polymerase at the *lac* promoter, in a transcriptionally repressed state, allowing rapid initiation of transcription once repressor is inactivated (14). In glucose–lactose medium, the *lac* transcription is induced quickly after the depletion of glucose. It is possible that CRP–cAMP may confer on the cell an effective way to switch from glucose to lactose utilization. Whether and how CRP–cAMP and *lac* repressor cooperate with each other *in vivo* remain to be determined.

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