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Expression of the glucose transporter gene, *ptsG*, is regulated at the mRNA degradation step in response to glycolytic flux in *Escherichia coli*

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We report a novel post-transcriptional control of the *ptsG* gene encoding the major glucose transporter IICB^{Glc}. We demonstrate that the level of IICB^{Glc} is markedly reduced when the glycolytic pathway is blocked by a mutation in either the *pgi* or *pfkA* gene encoding phosphoglucose isomerase or phosphofructokinase, respectively. This down-regulation of *ptsG* is not exerted at the transcriptional level. Both northern blot and S1 analyses demonstrate that the mutation dramatically accelerates the degradation of *ptsG* mRNA. The degradation of *ptsG* mRNA occurs in wild-type cells when α-methylglucoside, a non-metabolizable analog of glucose, is present in the medium. The addition of any one of the glycolytic intermediates downstream of the block prevents the degradation of *ptsG* mRNA. The rapid degradation of *ptsG* mRNA is eliminated when RNase E is thermally inactivated. We conclude that the glycolytic pathway controls *ptsG* expression by modulating RNase E-mediated mRNA degradation. This is the first instance in which the glycolytic flux has been shown to affect the expression of a specific gene through mRNA stability.

Keywords: glucose transporter/glycolysis/mRNA degradation/PTS/RNase E

Introduction

Glycolysis is a central metabolic pathway that is responsible for the production of numerous intermediary metabolites and energy in cells (Fraenkel, 1996). In bacteria, external glucose is transported into the cells and phosphorylated by the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) (Meadow *et al*., 1990; Postma *et al*., 1993; Saier, 1996). The phosphorylated glucose is then converted sequentially to pyruvate by a series of glycolytic enzymes. The PTS consists of two common cytoplasmic proteins, enzyme I and HPr, and an array of sugar-specific enzyme II complexes (EIIs). The glucose-specific EII (glucose transporter) consists of cytoplasmic protein IIA^{Glc} and membrane receptor IICB^{Glc} encoded by *crr* and *ptsG*, respectively. The phosphoryl group from PEP is transferred sequentially to enzyme I, to HPr, to EIIs and finally to glucose as it is translocated across the membrane.

In addition to the transport and phosphorylation of sugars, the PTS is involved in a variety of cellular physiological processes acting as a phospho-relay system. For example, IIA^{Glc} regulates both the transport of non-PTS carbohydrates and the activity of adenylate cyclase depending on its phosphorylation state (Meadow *et al*., 1990; Postma *et al*., 1993). The former process, called inducer exclusion, is totally responsible for the glucose–lactose diauxie that is a prototype of catabolite repression (Inada *et al*., 1996; Kimata *et al*., 1997). A fascinating recent discovery regarding the regulatory function of PTS is that IICB^{Glc}, depending on its phosphorylation state, interacts with Mlc to modulate the cellular localization and activity of this global repressor protein (Lee *et al*., 2000; Tanaka *et al*., 2000; Nam *et al*., 2001).

The expression of the *ptsG* gene encoding IICB^{Glc} is regulated by at least two global control systems at the transcriptional level (Kimata *et al*., 1997, 1998; Plumbridge, 1998). First, it is under positive control by cAMP receptor protein (CRP)–cAMP; therefore, *ptsG* expression is strongly dependent on this complex. Secondly, transcription of *ptsG* is regulated negatively by a global repressor Mlc. Recent studies have established that external glucose induces *ptsG* transcription by modulating the Mlc-mediated regulatory pathway (Kimata *et al*., 1998; Plumbridge, 1998). When glucose is transported into the cell, IICB^{Glc} is dephosphorylated and binds Mlc, resulting in sequestration of Mlc from the cytoplasm to the membrane (Lee *et al*., 2000; Tanaka *et al*., 2000; Nam *et al*., 2001).

While the transcriptional control of *ptsG* has been well documented, little is known about the post-transcriptional control of *ptsG* expression. We report here a new finding regarding the regulation of *ptsG* expression through mRNA stability. During a search for mutations that eliminate the inhibitory effect of glucose on *lac* operon expression, we found that *ptsG* expression was reduced dramatically when the glycolytic pathway was blocked. We showed that inhibition of the glycolytic pathway down-regulates *ptsG* expression by accelerating the degradation of *ptsG* mRNA. The destabilization of *ptsG* mRNA was eliminated when any one of the glycolytic intermediates downstream of the block was supplied in the growth medium or when RNase E was inactivated. Thus, the present study established that the flow of glycolysis controls *ptsG* expression by modulating RNase E-mediated mRNA degradation.

Results

A mutation in the pgi or pfkA gene eliminates glucose effect

The *lac* operon is expressed to only a small extent in wild-type *Escherichia coli* cells when both lactose and glucose

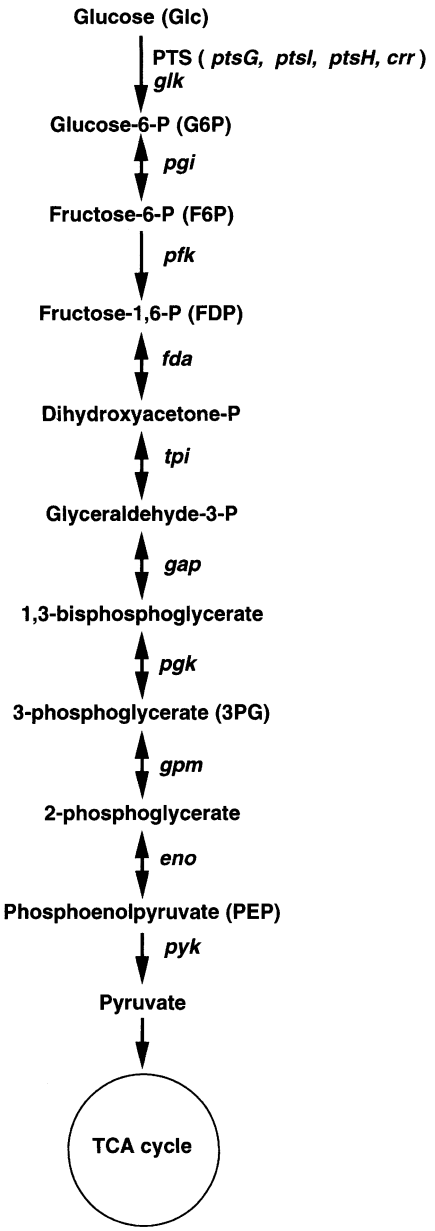

Fig. 1. The glycolytic pathway in *E.coli*. The phosphoenolpyruvate (PEP) phosphotransferase system (PTS) is the usual route of glucose uptake and phosphorylation in *E.coli*. Genes of the glycolytic enzymes responsible for each reaction are shown. Glucokinase encoded by *glk* may act to phosphorylate glucose generated internally (Fraenkel, 1996).

are present in the growth medium. This is because the transport of glucose into the cells by the PTS decreases the phosporylation state of IIA^{Glc}, which in turn leads to the inhibition of Lac permease by an inducer exclusion mechanism (Inada *et al*., 1996; Kimata *et al*., 1997). In order to gain further insight into the mechanism by which glucose affects *lac* operon expression, we searched for mutations that allow the expression of the *lac* operon in the presence of glucose. Wild-type cells form a white Lac^- colony on LB plates containing X-gal, glucose and lactose. We performed transposon mutagenesis by infecting wild-type W3110 cells with λ phage carrying Tn*5* *Km* (de Lorenzo *et al*., 1990). Mutants in which the *lac* operon was significantly expressed were selected as blue Lac^+

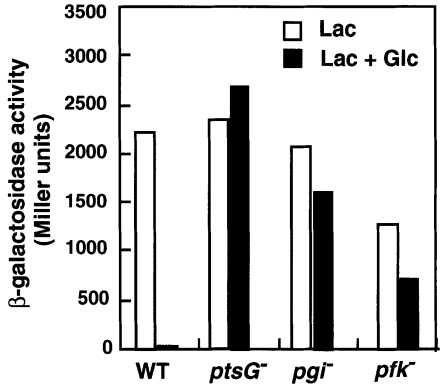

Fig. 2. The effect of glucose on *lac* operon expression is eliminated in *pgi* or *pfkA* mutants. PP6 (wild-type), KK27 (*ptsG*$^-$), KK52 (*pgi*$^-$) and KK51 (*pfkA*$^-$) cells were grown in LB medium with 1% lactose (Lac) or 0.5% glucose (Glc) + 0.5% lactose. Culture samples were taken at an OD_{600} of 0.6 and β-galactosidase activities were determined.

colonies on the indicator plates. One of the mutants that confer a Lac^+ phenotype in the presence of glucose was expected to carry the Tn*5* insertion in the *lacI* gene encoding Lac repressor. Mutation in the *crr* or *ptsG* gene should also give a Lac^+ phenotype because the IIA^{Glc}/$IICB^{Glc}$-dependent glucose uptake no longer occurs in *crr* or *ptsG* cells. In fact, DNA sequence analysis of the Lac^+ colonies revealed that this was exactly the case.

Interestingly, we also obtained Lac^+ colonies that carry the Tn*5* insertion in the other loci on which we focused in this study. DNA sequencing revealed that some Lac^+ colonies carry the Tn*5* insertion in either the *pgi* or *pfkA* gene encoding phosphoglucose isomerase or major phosphofructokinase (Pfk-1), respectively, both of which are glycolytic enzymes (Figure 1). To confirm that the Lac^+ phenotype was due to a single insertion of Tn*5* in the respective gene, the kanamycin-resistant phenotype of two representative clones was transferred by P1 transduction to the wild-type PP6 strain. The resulting *pgi* or *pfkA* strain was designated KK52 or KK51, respectively. To characterize the *pgi* and *pfkA* strains further, the effect of glucose on the β-galactosidase activity in these cells was determined in comparison with wild-type cells (Figure 2). In the presence of both lactose and glucose, the β-galactosidase activity was strongly inhibited through the inducer exclusion mechanism caused by the glucose transport in wild-type cells. On the other hand, β-galactosidase was expressed substantially in *pgi* and *pfkA* cells even in the medium with glucose, although its activity in the presence of lactose alone was moderately reduced compared with wild-type cells. *pgi* and *pfkA* mutation caused only a moderate reduction in the growth rate in the rich LB medium with or without glucose (data not shown). Thus, *pgi* and *pfkA* mutation eliminate the glucose effect, even if not completely. No inhibitory effect of glucose on β-galactosidase activity was observed in *ptsG* cells, as expected.

Expression of $IICB^{Glc}$ is markedly reduced in the pgi or pfkA background

How does mutaion of *pgi* or *pfkA* eliminate the inhibitory effect of glucose on *lac* expression? In principle, this could occur if these mutations reduce the expression of either

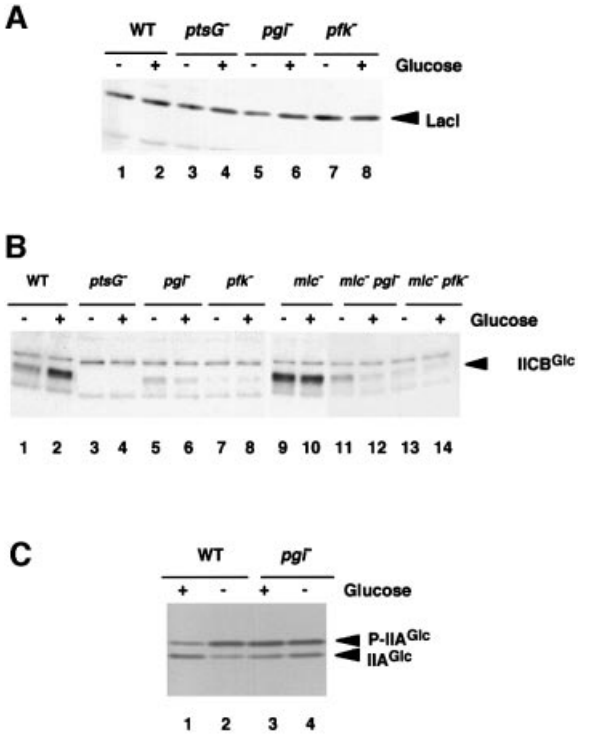

Fig. 3. Analyses of the proteins involved in the regulation of *lac* operon expression. (**A**) Western blot analysis of LacI. Cells were grown in LB medium with or without 1% glucose. Crude extracts equivalent to OD_{600} = 0.05 prepared from PP6 (lanes 1 and 2), KK27 (lanes 3 and 4), KK52 (lanes 5 and 6) and KK51 (lanes 7 and 8) were analyzed by western blot using anti-LacI. (**B**) Western blot analysis of $IICB^{Glc}$. Cells were grown in LB medium with or without 1% glucose. Crude extracts equivalent to OD_{600} = 0.01 prepared from PP6 (lanes 1 and 2), KK27 (lanes 3 and 4), KK52 (lanes 5 and 6), KK51 (lanes 7 and 8), KK32 (lanes 9 and 10), KK88 (lanes 11 and 12) and KK87 (lanes 13 and 14) were analyzed by western blot using anti-$IICB^{Glc}$. (**C**) Determination of the phosphorylation state of IIA^{Glc}. Cells were grown in LB medium with or without 1% glucose. Samples were prepared from PP6 (lanes 1 and 2) and KK52 (lanes 3 and 4), and analyzed by western blot using anti-IIA^{Glc} as described in Materials and methods.

LacI, IIA^{Glc} or $IICB^{Glc}$. To test this, we first analyzed the effect of *pgi* or *pfkA* mutation on the level of LacI by western blotting using an antibody raised against LacI. As shown in the Figure 3A, LacI expression was not affected significantly by *pgi* or *pfkA* mutation either in the presence or absence of glucose. Next, we determined the relative levels of $IICB^{Glc}$ in mutant cells compared with wild-type cells by western blot analysis using anti-$IICB^{Glc}$ antibody. The expression of $IICB^{Glc}$ was significantly induced by glucose in the wild-type cells (Figure 3B, lanes 1 and 2) while no $IICB^{Glc}$ was detected in the *ptsG* cells (Figure 3B, lanes 3 and 4). Interestingly, the level of $IICB^{Glc}$ was markedly reduced by the *pgi* or *pfkA* mutation especially in the presence of glucose (Figure 3B, lanes 5–8). Thus, it is highly possible that the $IICB^{Glc}$ level in these cells is not high enough to transport external glucose sufficiently; therefore, a significant fraction of IIA^{Glc} remains to be phosphorylated. In fact, the phosphorylation state of IIA^{Glc} was hardly affected by glucose in *pgi* cells (Figure 3C, lanes 3 and 4) while the presence of glucose in the growth medium stimulates dephosphorylation of P-IIA^{Glc} in wild-type cells (Figure 3C, lanes 1 and 2). Figure 3C also indicates that *pgi* or *pfkA* mutation does not affect IIA^{Glc} expresssion. Taken together, we conclude that mutation of *pgi* or *pfkA* mutation eliminates the effect of glucose on *lac* expression by reducing the expression of $IICB^{Glc}$.

Reduction of ptsG expression in pgi or pfkA cells is independent of both the CRP–cAMP and Mlc pathways

The next question is how the *pgi* or *pfkA* mutation reduces the expression of $IICB^{Glc}$. Since the transcription of *ptsG* is regulated both positively by CRP–cAMP and negatively by Mlc, these mutations could affect the expression of $IICB^{Glc}$ through these two global regulatory pathways. However, the CRP–cAMP system apparently is functional in *pgi* or *pfkA* cells because the expression of the *lac* operon, which is totally dependent on CRP–cAMP, is still significantly expressed in these cells (see Figure 2). This suggests that the reduction of $IICB^{Glc}$ expression in mutant cells may occur independently of CRP–cAMP. We also tested whether *pgi* or *pfkA* mutation reduces the expression of $IICB^{Glc}$ by enhancing the action of Mlc. If this were the case, $IICB^{Glc}$ should be highly expressed in the *mlc* background regardless of whether the *pgi* or *pfkA* mutation is present or not. However, expression of $IICB^{Glc}$ in *pgi mlc* (KK88) or *pfkA mlc* (KK87) cells was far less than that of the parent *mlc* cells (Figure 3B, lanes 9–14). Thus, the inhibitory effect of *pgi* or *pfkA* mutation on $IICB^{Glc}$ expression is essentially independent of the negative regulation of *ptsG* transcription by Mlc.

ptsG transcription occurs normally in pgi or pfkA cells

The results presented above raised the question of whether the down-regulation of *ptsG* by mutation of *pgi* or *pfkA* is achieved at the level of transcription. For this, we performed a quantitative S1 analysis using a 5′ DNA probe A (Figure 4A) and determined the relative levels of the 5′ *ptsG* transcript expressed in various cells. As expected, the abundance of the 5′ *ptsG* transcript increased upon addition of glucose in wild-type cells (Figure 4B, lanes 1 and 2) while it was highly expressed in the absence of glucose in *mlc* cells (Figure 4B, lane 3). The level of the 5′ *ptsG* transcript was reduced moderately in the presence of glucose (Figure 4B, lane 4). This is due to the reduction of both CRP and cAMP levels. Interestingly, *ptsG* transcription was induced normally by glucose in *pgi* cells and the relative abundance of the 5′ *ptsG* transcript was nearly identical to that of wild-type cells (Figure 4B, lanes 5 and 6). Similar results were also observed in *pfkA* cells although the induced level of the 5′ *ptsG* transcript was moderately reduced compared with wild-type cells (Figure 4B, lanes 7 and 8). These results indicate that $IICB^{Glc}$ could still modulate the localization of Mlc in response to the availability of glucose although its level is not sufficient to dephosphorylate P-IIA^{Glc}. The 5′ *ptsG* transcript was constitutively expressed in *mlc pgi* or *mlc pfkA* cells (Figure 4B, lanes 9–12). Thus, it is apparent that the 5′ portion of *ptsG* is transcribed normally in *pgi* or *pfkA* cells. These results strongly suggest that mutation of *pgi* or *pfkA* affects *ptsG* expression by some unknown mechanism(s) after transcription initiation.

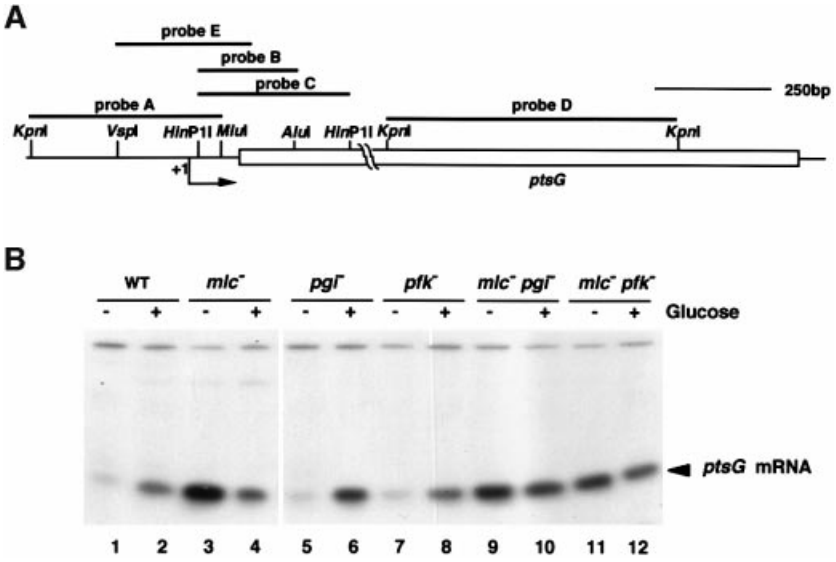

Fig. 4. Transcription of *ptsG* occurs normally in *pgi* or *pfkA* mutants. (**A**) Diagram of the *ptsG* region. The open bar represents the coding region for *ptsG*. The arrow denotes the start and direction of *ptsG* transcription. DNA fragments used as probes are shown above the map. The relevant restriction sites are shown. (**B**) S1 analysis of the 5′ portion of *ptsG* mRNA. Total RNAs (40 μg) prepared from PP6 (lanes 1 and 2), KK32 (lanes 3 and 4), KK52 (lanes 5 and 6), KK51 (lanes 7 and 8), KK88 (lanes 9 and 10) and KK87 (lanes 11 and 12) were subjected to S1 analysis using ^{32}P-labeled probe A.

pgi or pfkA mutation stimulates degradation of ptsG mRNA

In order to understand the mechanism underlying the down-regulation of *ptsG* by *pgi* or *pfkA* mutation, we analyzed the *ptsG* mRNA expressed in cells growing in the presence of glucose by northern blotting using DNA probe C (Figure 4A) corresponding to the 5′ portion of the *ptsG* gene. The full-length *ptsG* mRNA of ~1600 nucleotides was expressed in both wild-type and *mlc* cells (Figure 5A, lanes 1 and 2). Interestingly, *pgi* or *pfkA* mutation caused a dramatic decrease in the full-length *ptsG* mRNA, resulting in an extensive smear together with a short band of ~100 nucleotides on the northern blot (Figure 5A, lanes 3 and 4), suggesting that *ptsG* mRNA is degraded rapidly in *pgi* or *pfkA* cells. A similar smear of *ptsG* mRNA was also observed in the *mlc pgi* or *mlc pfkA* double mutant (Figure 5, lanes 5 and 6). When probe D corresponding to the 3′ portion of *ptsG* was used in northern blot analysis, the short band detected by the 5′ probe C was no longer observed while the full-length *ptsG* mRNA was detected (Figure 5B). This suggests that the short RNA band, which may represent a relatively stable degradation intermediate of *ptsG* mRNA, may be derived from the 5′ portion of *ptsG* mRNA. The 3′ end of this RNA was mapped just before the translation initiation codon of *ptsG* mRNA by S1 mapping experiment using the DNA probe B ^{32}P-labeled at its 3′ end (data not shown).

Selective effect of pgi or pfkA mutation on mRNA stability

To examine whether mutation of *pgi* or *pfkA* affects the expression and stability of other mRNAs, we performed northern blot analysis with the *crp* and *lac* mRNAs. We found that the stability of *crp* mRNA was not significantly affected by the *pgi* mutation (Figure 5C, left). Similarly, the *pgi* mutation did not significantly affect the stability and/or expression of *lac* mRNA (data not shown). Because mutation of *pgi* or *pfkA* caused a moderate reduction in the growth rate and some mRNAs such as *ompA* are known to be destabilized when the growth rate is reduced (Nilsson *et al*., 1984; Vytvytska *et al*., 1998), it is interesting to examine how mutation of *pgi* or *pfkA* affect the expression and stability of *ompA* mRNA. No dramatic degradation of *ompA* mRNA was observed in *pgi* cells, although the level of *ompA* mRNA was reduced moderately (Figure 5C, right). We conclude that the effect of mutation of *pgi* or *pfkA* on mRNA stability is selective rather than universal.

Half-life of ptsG mRNA is reduced dramatically in pgi cells

The results of northern blotting strongly suggest that the stability of *ptsG* mRNA must decrease significantly in *pgi* or *pfkA* cells. To evaluate the effect of mutation of *pgi* or *pfkA* on the stability of the *ptsG* mRNA more quantitatively, the chemical decay rate of the *ptsG* mRNAs in both wild-type and *pgi* cells was determined. Cells were grown to exponential phase in the presence of glucose, and rifamipicin was added to prevent further initiation of transcription. Cellular RNAs were isolated at various times after the addition of rifampicin, and these RNA samples were subjected to S1 nuclease assay using DNA probe E. As shown in Figure 6A, a dramatic increase in the decay of *ptsG* mRNA was observed in *pgi* cells. The radioactivity of the S1-resistant DNA bands was quantified to determine the rate of mRNA degradation (Figure 6B). The half-life of *ptsG* mRNA was estimated to be ~3.3 min in wild-type cells, while it was reduced to ~0.3 min in *pgi* cells. The reduction of the half-life of the 5′-most segment of *ptsG* mRNA in *pgi* cells was less significant when the S1 nuclease assay was performed using DNA probe A (data not shown). This is because the short degradation intermediate of *ptsG* mRNA produced in *pgi* cells was relatively stable.

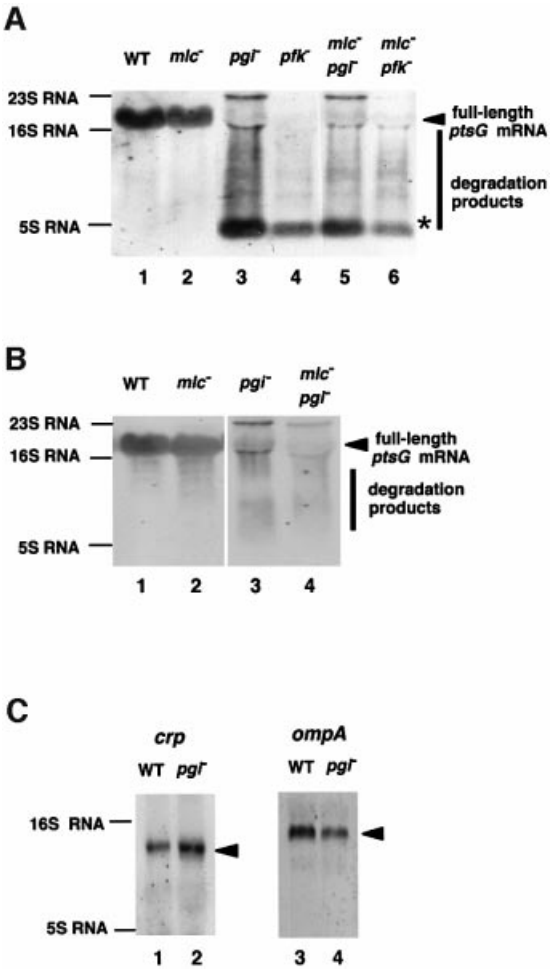

Fig. 5. The *ptsG* mRNA is degraded rapidly in *pgi* or *pfkA* mutants. (**A**) Northern blot analysis of the *ptsG* mRNA with the 5′ DNA probe. Total RNAs (40 μg) prepared from PP6 (lane 1), KK32 (lane 2), KK52 (lane 3), KK51 (lane 4), KK88 (lane 5) and KK87 (lane 6) grown in LB medium with 1% glucose were subjected to northern blot analysis using fluorescein-labeled probe C. (**B**) Northern blot analysis of *ptsG* mRNA with the 3′ DNA probe. Total RNAs (40 μg) prepared from PP6 (lane 1), KK32 (lane 2), KK52 (lane 3) and KK88 (lane 4) grown in LB medium with 1% glucose were subjected to northern blot analysis using fluorescein-labeled probe D. (**C**) Effect of the *pgi* mutation on the stability of *crp* and *ompA* mRNAs. Total RNAs (40 μg) prepared from PP6 (lanes 1 and 3) and KK52 (lanes 2 and 4) grown in LB medium with 1% glucose were subjected to northern blot analysis using fluorescein-labeled *crp* (lanes 1 and 2) or *ompA* (lanes 3 and 4) probes.

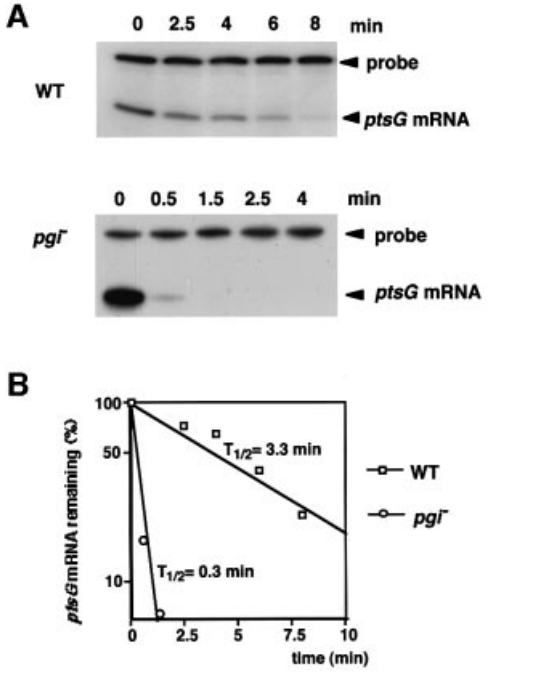

Fig. 6. Effect of the *pgi* mutation on *ptsG* mRNA stability analyzed by S1 assay. (**A**) *ptsG* mRNA decay in wild-type (upper) and *pgi*⁻ (lower) cells. PP6 and KK52 cells were grown in LB medium with 1% glucose to OD_{600} = 0.6 and treated with 0.2 mg/ml rifampicin. Cellular RNAs were prepared at the indicated times after the addition of rifampicin. Total RNAs of PP6 (40 μg) or KK52 (100 μg) were subjected to S1 analysis using ^{32}P-labeled probe E at its 5′ end. The exposure of the gel for autoradiography was much longer in *pgi* than in WT cells. (**B**) Semi-logarithmic plots of the radioactivity of S1-resistant DNA versus time. The S1-resistant DNA bands were quantified using the Bioimage Analyzer BAS2000 (Fuji). The half-life ($t_{1/2}$) of *ptsG* mRNA was determined based on these plots.

Exogenous glycolytic intermediates stabilize ptsG mRNA in the pgi or pfkA background

Because the block of the glycolytic pathway is expected to produce a variety of metabolic stresses, there are several possible factors that lead to the rapid degradation of *ptsG* mRNA in *pgi* or *pfkA* cells. For example, the missing protein, phosphoglucose isomerase or phosphofructokinase, in the mutant cells could be involved in the stabilization of *ptsG* mRNA. Secondly, the accumulation of glucose-6-phosphate (G6P) or fructose-6-phosphate (F6P) due to the mutation of *pgi* or *pfkA* could produce a signal for the destabilization of *ptsG* mRNA. Thirdly, deficiency of a single or several glycolysis intermediates after the block point could be responsible for the destabilization of *ptsG* mRNA. Finally, inhibition of the glycolytic pathway should affect downstream metabolic pathways such as the tricarboxylic acid (TCA) cycle, which may produce a signal for the destabilization of *ptsG* mRNA. To test these possibilities, we first examined the effect of α-methylglucoside on the stability of *ptsG* mRNA in wild-type cells. The normal glycolytic pathway would be inhibited in the presence of α-methylglucoside because this non-metabolizable glucose analog is transported and phosphorylated by the glucose PTS but not metabolized further. We found that the degradation of *ptsG* mRNA was stimulated by adding α-methylglucoside to wild-type cells as in the case of *pgi* or *pfkA* cells (Figure 7A). This suggests that the lack of phosphoglucose isomerase or phosphofructokinase itself is not responsible for the rapid degradation of *ptsG* mRNA. We then examined whether the addition of any of the glycolytic intermediates to the growth medium affects the stability of *ptsG* mRNA in *mlc pgi* or *mlc pfkA* double mutant cells (Figure 7B and C). The addition of F6P stabilized the *ptsG* mRNA in *pgi* but not in *pfkA* cells, while the addition of fructose-1,6-diphosphate (FDP) stabilized the *ptsG* mRNA in both cell types. These results suggest that accumulation of G6P or F6P may not be responsible for the destabilization of *ptsG* mRNA. Interestingly, all intermediates tested except G6P could restore the stability of *ptsG* mRNA in *pgi* cells (Figure 7B). Similarly, intermediates downstream of FDP could stabilize the *ptsG* mRNA in the *mlc pfkA* double mutant cells (Figure 7C). It should be noted that

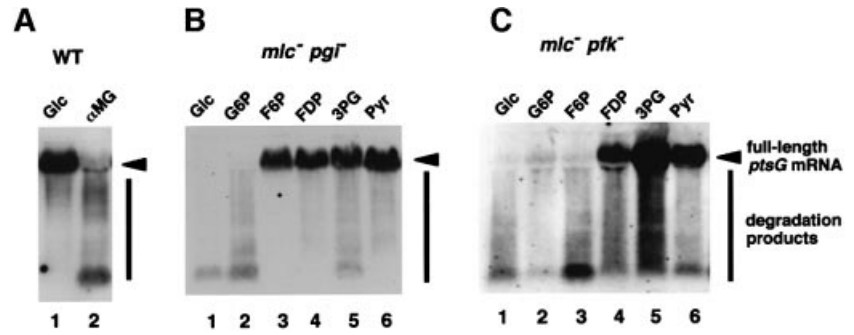

Fig. 7. Effect of the addition of metabolic intermediates or analogs on the stability of *ptsG* mRNA. PP6 (**A**), KK88 (**B**) and KK87 (**C**) cells were grown in LB medium with 1% of the following compounds: Glc, glucose; G6P, glucose-6-phosphate; αMG, α-methylglucoside; F6P, fructose-6-phosphate; FDP, fructose-1,6-diphosphate; 3PG, 3-phosphoglycerate; Pyr, pyruvate. Total RNAs (40 μg) were subjected to northern blotting with ^{32}P-labeled probe C.

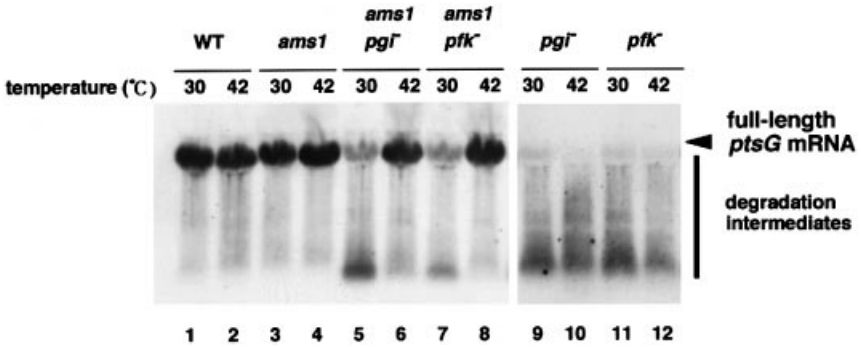

Fig. 8. Inactivation of RNase E stabilizes *ptsG* expression in *pgi* or *pfkA* mutants. PP6 (lanes 1 and 2), KK65 (lanes 3 and 4), KK69 (lanes 5 and 6), KK67 (lanes 7 and 8), KK52 (lanes 9 and 10) and KK51 (lanes 11 and 12) were grown in LB medium with 1% glucose (Glc) at 30°C. At OD_{600} = 0.5, the temperature was shifted to 42°C (lanes 2, 4, 6, 8, 10 and 12) or kept at 30°C (lanes 1, 3, 5, 7, 9 and 11). Cellular RNAs were prepared after the incubation was continued for 10 min at the indicated temperatures. Total RNAs (40 μg) were subjected to northern blotting with ^{32}P-labeled probe C.

pyruvate, which is located the most downstream of the glycolytic pathway could stabilize the *ptsG* mRNA in the mutant cells. Taken together, we conclude that deficiency of any of the glycolysis intermediates may not be responsible for the destabilization of *ptsG* mRNA in *pgi* or *pfkA* cells.

The rapid degradation of ptsG mRNA in pgi or pfkA cells is prevented by inactivation of RNase E

Because RNase E is the major ribonuclease responsible for general mRNA turnover (Kushner, 1996; Cohen and McDowall, 1997), we examined the effect of inactivation of RNase E on *ptsG* mRNA degradation. For this, we constructed three isogenic strains carrying a temperature-sensitive *rne* allele: KK65 (*ams-1*), KK69 (*pgi ams-1*) and KK67 (*pfkA ams-1*). Cells were grown at 30°C to mid-logarithmic phase in the presence of glucose and the temperature was shifted to 42°C to inactivate RNase E. Total RNAs were isolated before and 10 min after the temperature shift, and RNA samples were subjected to northern blot analysis using DNA probe C. The temperature shift from 30 to 42°C moderately increased the level of *ptsG* mRNA in *ams-1* cells, presumably due to the inactivation of RNase E (Figure 8, lanes 3 and 4), but not in wild-type cells (Figure 8, lanes 1 and 2). Marked degradation of *ptsG* mRNA was observed at 30°C in cells carrying both *ams-1* and *pgi*, or *pfkA ams-1* mutations (Figure 8, lanes 5 and 7) as in the case of *pgi* cells (Figure 8, lane 9). However, the rapid degradation of *ptsG* mRNA in the *ams-1* cells was inhibited when RNase E was inactivated by shifting to 42°C (Figure 8, lanes 6 and 8). The temperature shift from 30 to 42°C did not affect the degradation pattern of *ptsG* mRNA when cells carry only the *pgi* or *pfkA* mutation (Figure 8, lanes 9–12). These findings indicate that RNase E is responsible for the rapid degradation of *ptsG* mRNA when the glycolytic pathway is blocked.

Discussion

The PTS catalyzes the transport and phosphorylation of a number of carbohydrates in enteric bacteria and the phosphorylated sugars are metabolized by a series of chain reactions in which the glycolytic pathway always acts as a trunk route (Meadow *et al*., 1990; Postma *et al*., 1993; Saier, 1996). Thus, both the PTS and glycolysis play a central role in intermediary sugar metabolism. The expression of genes encoding various PTS components appears to be regulated in a complex and dynamic fashion to meet various physiological requirements. Transcription

of several PTS genes including *ptsG* has been shown to be under the control of both CRP–cAMP and Mlc (Kimata *et al*., 1997, 1998; Plumbridge, 1998). However, little is known about post-transcriptional regulation of PTS genes. We have demonstrated here that the expression of *ptsG* encoding a major glucose transporter is regulated at the level of mRNA degradation in response to the glycolytic flux. Our findings can be summarized as follows: (i) block of the glycolytic pathway causes a dramatic reduction in the level of $IICB^{Glc}$; (ii) this effect is independent of both CRP–cAMP and Mlc regulatory circuits and not exerted at the step of transcription initiation; (iii) the reduction in the $IICB^{Glc}$ level is due to a dramatic increase in the degradation rate of *ptsG* mRNA; (iv) α-methylglucoside, a non-metabolizable glucose analog, causes rapid degradation of *ptsG* mRNA in wild-type cells; (v) the presence of any glycolysis intermediates downstream of the block in growth medium could stabilize the *ptsG* mRNA in the *pgi* or *pfkA* mutant cells; and (vi) the destabilization of *ptsG* mRNA no longer occurs when RNase E is thermally inactivated in cells carrying a temperature-sensitive *rne* allele. These results led us to conclude that the block of the glycolytic pathway prevents *ptsG* expression by accelerating the degradation of *ptsG* mRNA and that RNase E is responsible for the rapid degradation of *ptsG* mRNA. Thus, we demonstrate a clear link between the carbon flux in central metabolism and the stability of a specific mRNA.

The degradation of mRNA is an important step for controlling gene expression because it determines, in part, the cellular concentration of mRNA and therefore the capacity for protein synthesis (Belasco, 1993; Grunberg-Manago, 1999; Rauhut and Klug, 1999). The longevity of bacterial mRNAs varies among transcripts and the rate of degradation of individual mRNAs can vary in response to changes in the environmental conditions either selectively or pleiotropically (Belasco, 1993). The best known example of this is the growth-rate dependent regulation of certain mRNA species in *E.coli*. It is well known that the long-lived *ompA* mRNA encoding the major outer membrane protein A decreases under conditions of slow cell growth (Nilsson *et al*., 1984; Vytvytska *et al*., 1998). The initial cleavage in the 5′-untranslated region of the *ompA* mRNA during slow growth was attributed to RNase E (Lundberg *et al*., 1990). Another environmental signal that affects mRNA stability in *E.coli* cells is oxygen availability. A general mRNA stabilization coupled with a decrease in RNA synthesis occurs during anaerobic slow growth, suggesting that oxygen availability somehow regulates RNases (Georgellis *et al*., 1993). Any environmental stimulus such as growth rate or oxygen availability should affect the flux in various metabolic pathways. However, little is known about whether a specific change in a certain metabolic pathway affects the stability of individual mRNAs. We showed here that *ptsG* mRNA becomes extremely unstable when glycolysis is blocked. Although the inhibition of the glycolytic pathway by *pgi* or *pfkA* mutation caused a moderate reduction in the growth rate, the dramatic effect on mRNA stability apparently is rather selective for *ptsG* mRNA because the stability of other mRNAs, including that for *ompA*, was not significantly affected by *pgi* or *pfkA* mutation. The present study has given a clear example where genetic and/or nutritional alterations in the central metabolism cause a dramatic change in stability of a specific mRNA to regulate its expression.

How does the inhibition of the glycolytic pathway enhance the degradation of *ptsG* mRNA? We showed that RNase E is certainly responsible for the rapid degradation of *ptsG* mRNA. Our data indicate that neither accumulation nor shortage of any of the intermediate metabolites of glycolysis is responsible for the rapid degradation of *ptsG* mRNA by RNase E. It is likely that inhibition of the glycolytic pathway may somehow produce a signal to stimulate the RNase E-dependent degradation of *ptsG* mRNA by affecting downstream metabolic pathways such as the TCA cycle. How the inhibition of the glycolytic pathway activates RNase E-mediated degradation of *ptsG* mRNA remains to be resolved.

RNase E encoded by *rne/ams* has an important role in the degradation of a variety of mRNAs in *E.coli* cells (Cohen and McDowall, 1997; Ow *et al*., 2000). It has been established that RNase E exists as a macromolecular complex called a degradosome, a multienzyme RNA degradation complex that also contains PNPase, RhlB helicase, enolase and other minor components (Carpousis *et al*., 1994; Py *et al*., 1994, 1996; Miczak *et al*., 1996). PNPase, one of two known 3′ to 5′ exonucleases, is believed to have an important role in degrading products generated by RNase E. The role of RhlB, a member of the DEAD-box family of ATP-dependent RNA helicases, would be to unwind the secondary structure to permit access by RNase E and/or PNPase (Coburn and Mackie, 1999). The role of enolase in RNA degradation remains a completely mystery. It would be interesting to test whether any of these components of the degradosome plays some role in the regulation of the RNase E-mediated degradation of *ptsG* mRNA.

The pathway for mRNA degradation apparently involves multiple steps and additional *trans*-acting factors. For example, host factor I (Hfq), originally recognized for its function in phage Qβ replication, binds to *ompA* mRNA in a growth rate-dependent fashion and stimulates its decay by interfering with ribosome binding (Vytvytska *et al*., 1998, 2000). Another example is CsrA, originally identified as a negative regulator of glycogen biosynthesis genes such as *glgC* and now known to be involved in pleiotropic regulation of central carbohydrate metabolism (Liu *et al*., 1997; Romeo, 1998). CsrA has been shown to enhance the decay of *glgC* mRNA by acting as an RNA-binding protein (Liu and Romeo, 1997). It will be interesting to determine whether Hfq and/or CsrA are involved in the regulation of the RNase E-mediated degradation of *ptsG* mRNA.

What is the physiological relevance of the modulation of *ptsG* mRNA stability by the central carbon flux? One situation in which the glycolysis flux decreases would be shortage of carbohydrate in the growth medium. In fact, we observed that the degradation of *ptsG* mRNA is partially stimulated when wild-type cells are forced to use only amino acids as carbon source (our unpublished results). The reduced stability of *ptsG* mRNA along with the negative regulation of *ptsG* transcription by Mlc is useful to prevent unnecessary synthesis of transporter protein when its substrate is absent. On the other hand, the stabilization of *ptsG* mRNA certainly allows the cells to

Table I. Bacterial strains used in this study

Strain/ plasmid	Relevant genotype and property	Source
PP6	wild type	Kimata *et al*. (1998)
IT1165	W3110 *pfkA*::Tn*5*	this study
IT1320	W3110 *pgi*::Tn*5*	this study
IT1168	W3110 *ptsG*::Tn*5*	Kimata *et al*. (1997)
KK27	PP6 *ptsG*::Tn*5*	Tanaka *et al*. (2000)
KK32	PP6 *mlc1157*::Tn*10*	Kimata *et al*. (1998)
KK51	PP6 *pfkA*::Tn*5*	this study
KK52	PP6 *pgi*::Tn*5*	this study
KK87	PP6 *pfkA*::Tn*5 mlc1157*::Tn*10*	this study
KK88	PP6 *pgi*::Tn*5 mlc1157*::Tn*10*	this study
GW10	W3110 *zce*-726::Tn*10*	Wachi *et al*. (1997)
GW20	W3110 *ams1 zce*-726::Tn*10*	Wachi *et al*. (1997)
KK64	PP6 *zce*-726::Tn*10*	this study
KK65	PP6 *ams1 zce*-726::Tn*10*	this study
KK66	PP6 *pfkA*::Tn*5 zce*-726::Tn*10*	this study
KK67	PP6 *pfkA*::Tn*5 ams1 zce*-726::Tn*10*	this study
KK68	PP6 *pgi*::Tn*5 zce*-726::Tn*10*	this study
KK69	PP6 *pgi*::Tn*5 ams1 zce-726::*Tn*10*	this study

synthesize IICB^{Glc} rapidly when *ptsG* transcription is induced through inactivation of Mlc in response to glucose availability.

The discovery of a close link between central metabolism and mRNA stability has raised a number of questions and opened up new avenues for future studies. How does inhibition of the glycolytic pathway produce a signal that leads to the rapid degradation of *ptsG* mRNA mediated by RNase E? Is the regulation of *ptsG* mRNA degradation mediated by *trans*-acting factors such as Hfq and CsrA/CsrB? Are there any other mRNAs that are destabilized by the inhibition of the glycolytic pathway? What are the structural determinants of *ptsG* mRNA stability recognized by RNase E and/or some other factors? Does the inhibition of the glycolytic pathway affect the level of the components of the degradosome? Do mutations in other glycolytic genes affect the stability of *ptsG* mRNA? Many of these questions would be testable experimentally. Regarding the last question, it should be noted that mutation of *ts8*, an allele of *fda* encoding another glycolytic enzyme fructose-1,6-diphosphate aldolase, is reported to inhibit stable RNA synthesis at the non-permissive temperature (Singer *et al*., 1991). It will certainly be interesting to test the *fda* mutation on *ptsG* mRNA.

Materials and methods

Bacterial strains and growth conditions

The *E.coli* K-12 strains used in this study are listed in Table I. PP6 was used as a parent wild-type strain. IT1320 (W3110 *pgi*::Tn*5*) and IT1165 (W3110 *pfkA*::Tn*5*) were obtained by random insertion of Tn*5* from W3110. The *pgi*::Tn*5* region of IT1320 or the *pfkA*::Tn*5* region of IT1165 was transferred to PP6 by P1 transduction to obtain KK52 and KK51, respectively. The *mlc*::Tn*10* region of KK32 (PP6 *mlc1157*::Tn*10*) was transferred to KK52 and KK51 by P1 transduction to obtain KK88 and KK87, respectively. The *zce*-726::Tn*10* region of GW10 (Wachi *et al*., 1997) was transferred to PP6, KK52 and KK51 by P1 transduction to obtain KK64, KK68 and KK66, respectively. The *ams-1 zce*-726::Tn*10* region of GW20 (Wachi *et al*., 1997) was transferred to PP6, KK52 and KK51 by P1 transduction to obtain KK65, KK69 and KK67, respectively. Cells were grown aerobically at 37°C in Luria–Bertani broth (LB) medium (Miller, 1972). Antibiotics were used at the following concentrations: ampicillin (50 μg/ml), kanamycin (50 μg/ml), tetracycline (15 μg/ml) and chloramphenicol (15 μg/ml). Bacterial growth was monitored by determining the optical density at 600 nm.

β-galactosidase assay

β-galactosidase activity was determined according to the method of Miller (1972).

Western blotting

Bacterial cells grown in LB medium containing appropriate antibiotic(s) were harvested at OD_{600} = 0.6 and suspended in 100 μl of SDS–PAGE loading buffer (62.5 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.1% bromophenol blue). The sample was heated at 100°C for 5 min. The indicated amounts of total cellular proteins were subjected to 12% acrylamide–0.1% SDS gel electrophoresis and transferred to an Immobilon membrane (Millipore). The polypeptides detected by the antibodies were visualized by the enhanced chemiluminescence (ECL) system (Pharmacia). The anti-LacI, anti-IIB^{Glc} and anti-IIA^{Glc} polyclonal antibodies have been described previously (Tanaka *et al*., 1999, 2000; Abo *et al*., 2000).

Determination of the phosphorylation state of IIA^{Glc}

The phosphorylation state of IIA^{Glc} in cells was determined by the alkali-freeze method (Takahashi *et al*., 1998). Cells were grown to an OD_{600} of 0.6. Culture samples of 0.2 ml were taken and immediately killed by adding with 20 μl of 10 M NaOH followed by vigorous vortexing for 10 s. Then, 180 μl of 3 M sodium acetate pH 5.2 and 1 ml of ethanol were added. The mixture was chilled at –70°C for 10 min and centrifuged at 12 000 *g* for 10 min at 4°C. The resulting pellet was rinsed with 70% ethanol and dissolved in 100 μl of SDS sample buffer (2% SDS, 5% 2-mercaptoethanol, 62.5 mM Tris–HCl pH 8.0, 10% glycerol, 0.1% bromophenol blue). Then 20 μl of sample was subjected to SDS–12% polyacrylamide gel electrophoresis and analyzed by western blotting using polyclonal anti-IIA^{Glc} antibody.

S1 nuclease analysis

The S1 nuclease assay was performed as described previously (Aiba *et al*., 1981). Total cellular RNAs were isolated from cells grown in LB medium to an OD_{600} of 0.6. The 411 bp *Kpn*I–*Mlu*I DNA fragment (probe A) ^{32}P-labeled at the *Mlu*I 5′ end was used as a DNA probe to determine the relative level of the 5′ most portion of *ptsG* mRNA (Figure 4). The 283 bp *Vsp*I-PG3 (PG3; GGAGCACTCTCAATTATGTTTAAGAATGC) DNA fragment (probe E) ^{32}P-labeled at the PG3 5′ end, obtained by PCR, was used for determination of the half-life of *ptsG* mRNA. The 204 bp *Hin*P1I–*Alu*I DNA fragment (probe B) ^{32}P-labeled at the *Hin*P1I 3′ end was used to determine the 3′ end of the short degradation intermediate of *ptsG* mRNA. The DNA fragments were obtained by PCR amplification, except probes A and B, which were derived from purified plasmid pIT499 (Kimata *et al*., 1997). The DNA probe and total RNA were hybridized and treated with 40 U of S1 nuclease at 37°C for 15 min. The reaction products were analyzed on an 8% polyacrylamide–8 M urea gel.

Northern blot analysis

The total RNAs were resolved by 1.0% agarose gel electrophoresis in the presence of formamide and blotted onto Hybond-N$^+$ membrane (Amersham Pharmacia Biotech) as described (Sambrook *et al*., 1989). The DNA probes were labeled with fluorescein-11-dUTP by random priming (Amersham Pharmacia Biotech). The 571 bp *Kpn*I–*Kpn*I fragment (probe C) corresponding to the 3′ portion of the *ptsG* structural gene and the 326 bp *Hin*P1I–*Hin*P1I fragment (probe E) containing the 5′ portion of the *ptsG* gene were used as the 3′ and 5′ DNA probes, respectively. The 725 bp *Nru*I–*BamH*I DNA fragment containing the *ompA* structural gene and the 738 bp *Hin*dIII–*Eco*RV DNA fragment containing the *crp* gene were used as probes for *ompA* and *crp* mRNAs, respectively. The DNA fragments used were obtained by PCR amplification. The membrane was hybridized with the probes and washed. The signals were produced by addition of the anti-fluorescein alkaline phosphatase conjugate and CDP-Star (Amersham Pharmacia Biotech) to the membrane and visualized by exposure to films.

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References

Abo,T., Inada,T., Ogawa,K. and Aiba,H. (2000) SsrA-mediated tagging and proteolysis of LacI and its role in regulation of the *lac* operon. *EMBO J.*, **19**, 3762–3769.

Aiba,H., Adhya,S. and de Crombrugghe,B. (1981) Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. *J. Biol. Chem.*, **256**, 11905–11910.

Belasco,J. (1993) mRNA degradation in proprokaryotic cells: an overview. In Belasco,J. and Brawerman,G. (eds), *Control of Messenger RNA Stability*. Academic Press, New York, NY, pp. 3–12.

Carpousis,A.J., Van Houwe,G., Ehretsmann,C. and Krisch,H.M. (1994) Copurification of *E.coli* RNAase E and PNPase: evidence for a specific association between two enzymes important in RNA processing and degradation. *Cell*, **76**, 889–900.

Coburn,G.A. and Mackie,G.A. (1999) Degradation of mRNA in *Escherichia coli*: an old problem with some new twists. *Prog. Nucleic Acid Res. Mol. Biol.*, **62**, 55–108.

Cohen,S.N. and McDowall,K.J. (1997) RNase E: still a wonderfully mysterious enzyme. *Mol. Microbiol.*, **23**, 1099–1106.

de Lorenzo,V., Herrero,M., Jakubzik,U. and Timmis,K.N. (1990) Mini-Tn*5* transposon derivatives for insertion mutagenesis, promoter probing and chromosomal insertion of cloned DNA in gram-negative eubacteria. *J. Bacteriol.*, **172**, 6568–6572.

Fraenkel,D.G. (1996) Glycolysis. In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium*: *Cellular and Molecular Biology*. ASM Press, Washington, DC, pp. 189–205.

Georgellis,D., Barlow,T., Arvidson,S. and von Gabain,A. (1993) Retarded RNA turnover in *Escherichia coli*: a means of maintaining gene expression during anaerobiosis. *Mol. Microbiol.*, **9**, 375–381.

Grunberg-Manago,M. (1999) Messenger RNA stability and its role in control of gene expression in bacteria and phages. *Annu. Rev. Genet.*, **33**, 193–227.

Inada,T., Kimata,K. and Aiba,H. (1996) Mechanism responsible for glucose–lactose diauxie in *Escherichia coli*: challenge to the cAMP model. *Genes Cells*, **1**, 293–301.

Kimata,K., Takahashi,H., Inada,T., Postma,P. and Aiba,H. (1997) cAMP receptor protein–cAMP plays a crucial role in glucose–lactose diauxie by activating the major glucose transporter gene in *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **94**, 12914–12919.

Kimata,K., Inada,T., Tagami,H. and Aiba,H. (1998) A global repressor (Mlc) is involved in glucose induction of the *ptsG* gene encoding major glucose transporter in *Escherichia coli*. *Mol. Microbiol.*, **29**, 1509–1519.

Kushner,S.R. (1996) mRNA decay. In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium*: *Cellular and Molecular Biology*. ASM Press, Washington, DC, pp. 849–860.

Lee,S.J., Boos,W., Bouche,J.P. and Plumbridge,J. (2000) Signal transduction between a membrane-bound transporter, PtsG and a soluble transcription factor, mlc, of *Escherichia coli*. *EMBO J.*, **19**, 5353–5361.

Lundberg,U., von Gabain,A. and Melefors,Ö. (1990) Cleavages in the 5′ region of the *ompA* and *bla* mRNA control stability: studies with an *E.coli* mutant altering mRNA stability and a novel endoribonuclease. *EMBO J.*, **9**, 2731–2741.

Liu,M.Y. and Romeo,T. (1997) The global regulator CsrA of *Escherichia coli* is a specific mRNA-binding protein. *J. Bacteriol.*, **179**, 4639–4642.

Liu,M.Y., Gui,G., Wei,B., Preston,J.F., Oakford,L., Yuksel,U., Giedroc,D.P. and Romeo,T. (1997) The RNA molecule CsrB binds to the global regulatory protein CsrA and antagonizes its activity in *Escherichia coli*. *J. Biol. Chem.*, **272**, 17502–17510.

Meadow,N.D., Fox,D.K. and Roseman,S. (1990) The bacterial phosphoenolpyruvate:glycose phosphotransferase system. *Annu. Rev. Biochem.*, **59**, 497–542.

Miczak,A., Kaberdin,V.R., Wei,C.L. and Lin-Chao,S. (1996) Proteins associated with RNase E in a multicomponent ribonucleolytic complex. *Proc. Natl Acad. Sci. USA*, **93**, 3865–3869.

Miller,J. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Nam,T.W. *et al*. (2001) The *Escherichia coli* glucose transporter enzyme IICB(Glc) recruits the global repressor Mlc. *EMBO J.*, **20**, 491–498.

Nilsson,G., Belasco,J.G., Cohen,S.N. and von Gabain,A. (1984) Growth-rate dependent regulation of mRNA stability in *Escherichia coli*. *Nature*, **312**, 75–77.

Ow,M.C., Liu,Q. and Kushner,S.R. (2000) Analysis of mRNA decay and rRNA processing in *Escherichia coli* in the absence of RNase E-based degradosome assembly. *Mol. Microbiol.*, **38**, 854–866.

Plumbridge,J. (1998) Expression of *ptsG*, the gene for the major glucose PTS transporter in *Escherichia coli*, is repressed by Mlc and induced by growth on glucose. *Mol. Microbiol.*, **29**, 1053–1063.

Postma,P.W., Lengeler,J.W. and Jacobson,G.R. (1993) Phospho-enolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol. Rev.*, **57**, 543–594.

Py,B., Causton,H., Mudd,E.A. and Higgins,C.F. (1994) A protein complex mediating mRNA degradation in *Escherichia coli*. *Mol. Microbiol.*, **14**, 717–729.

Py,B., Higgins,C.F., Krisch,H.M. and Carpousis,A.J. (1996) A DEAD-box RNA helicase in the *Escherichia coli* RNA degradosome. *Nature*, **381**, 169–172.

Rauhut,R. and Klug,G. (1999) mRNA degradation in bacteria. *FEMS Microbiol. Rev.*, **23**, 353–370.

Romeo,T. (1998) Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule CsrB. *Mol. Microbiol.*, **29**, 1321–1330.

Saier,M.H.,Jr, Ramseier,T.M. and Reizer,J. (1996) Regulation of carbon utilization. In Neidhardt,F.C. (ed.), *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*. ASM Press, Washington, DC, pp. 1325–1343.

Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Singer,M., Rossmiessl,P., Cali,B.M., Liebke,H. and Gross,C.A. (1991) The *Escherichia coli ts8* mutation is an allele of *fda*, the gene encoding fructose-1,6-diphosphate aldolase. *J. Bacteriol.*, **173**, 6242–6248.

Takahashi,H., Inada,T., Postma,P. and Aiba,H. (1998) CRP down-regulates adenylate cyclase activity by reducing the level of phosphorylated IIA(Glc), the glucose-specific phosphotransferase protein, in *Escherichia coli*. *Mol. Gen. Genet.*, **259**, 317–326.

Tanaka,Y., Kimata,K., Inada,T., Tagami,H. and Aiba,H. (1999) Negative regulation of the *pts* operon by Mlc: mechanism underlying glucose induction in *Escherichia coli*. *Genes Cells*, **4**, 391–399.

Tanaka,Y., Kimata,K. and Aiba,H. (2000) A novel regulatory role of glucose transporter of *Escherichia coli*: membrane sequestration of a global repressor Mlc. *EMBO J.*, **19**, 5344–5352.

Vytvytska,O., Jakobsen,J.S., Balcunaite,G., Andersen,J.S., Baccarini,M. and von Gabain,A. (1998) Host factor I, Hfq, binds to *Escherichia coli ompA* mRNA in a growth rate-dependent fashion and regulates its stability. *Proc. Natl Acad. Sci. USA*, **95**, 14118–14123.

Vytvytska,O., Moll,I., Kaberdin,V.R., von Gabain,A. and Blasi,U. (2000) Hfq (HF1) stimulates *ompA* mRNA decay by interfering with ribosome binding. *Genes Dev.*, **14**, 1109–1118.

Wachi,M., Umitsuki,G. and Nagai,K. (1997) Functional relationship between *Escherichia coli* RNase E and the CafA protein. *Mol. Gen. Genet.*, **253**, 515–519.

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