Mechanism responsible for glucose–lactose diauxie in *Escherichia coli*: challenge to the cAMP model

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Abstract

***Background*: The inhibition of β-galactosidase expression in glucose–lactose diauxie is a typical example of the glucose effect in *Escherichia coli*. It is generally believed that glucose exerts its effect at least partly by reducing the intracellular cAMP level. However, there is no direct evidence that the inhibitory effect of glucose on the expression of the *lac* operon is mediated by a reduction of the cAMP level in the glucose–lactose system.**

***Results*: To examine the roles of cAMP and the cAMP receptor protein (CRP) in the glucose effect, the intracellular levels of these factors were determined during diauxic growth in a glucose–lactose medium. We found that the levels of cAMP and CRP in a lactose-grown phase were not higher than those in a glucose-grown phase, although the cAMP levels increased transiently during the lag phase. The addition of exogenous cAMP eliminated diauxic growth but did not eliminate glucose repression. Glucose repression and diauxie were observed in cells which lack cAMP but produce a cAMP-independent CRP. In addition, inactivation of the *lac* repressor by the disruption of the *lacI* gene or the addition of IPTG, eliminated glucose repression.**

***Conclusion*: We conclude that the repression of β-galactosidase expression by glucose is not due to the reduction of the cAMP-CRP level but due to an inducer exclusion mechanism which is mediated by the phosphoenolpyruvate-dependent sugar phosphotransferase system.**

Introduction

The inhibitory effect of glucose on the synthesis of catabolic enzymes, termed the glucose effect, has been extensively studied, in particular on the *Escherichia coli lac* operon and one of its products, β-galactosidase (reviewed by Magasanik 1970; Pastan & Adhya 1976; Ullmann & Danchin 1983; Magasanik & Neidhardt 1987). The *lac* operon of *Escherichia coli* has long been a paradigm for understanding how gene expression in bacteria is controlled in response to nutrient conditions. Early studies on the regulation of the *lac* operon led to the discovery of the dual control system that consists of negative and positive regulation by the *lac* repressor and the CRP-cAMP complex, respectively (reviewed by Reznikoff & Abelson 1978; Reznikoff 1992).

Although the mechanism of glucose effects is not yet fully understood, glucose would be expected to inhibit the expression of the *lac* operon ultimately by modulating the activity of CRP-cAMP and/or that of *lac* repressor. First, glucose is known to lower intracellular cAMP under certain conditions (Makman & Sutherland 1965; Pastan & Perlman 1970). It is believed that glucose lowers cAMP levels by decreasing the level of the phosphorylated form of enzyme IIA^{Glc} which is thought to be an activator of adenylate cyclase (reviewed by Magasanik & Neidhardt 1987; Saier Jr 1989; Postma *et al.* 1993). Second, glucose has been shown to lower the intracellular CRP level through the autoregulatory loop of the *crp* gene (Hanamura *et al.* 1992; Ishizuka *et al.* 1993, 1994). Third, glucose inhibits the uptake of the inducer, resulting in an increase in the concentration of the active repressor (reviewed by Magasanik & Neidhardt 1987; Roseman & Meadow 1990; Saier Jr 1989; Postma *et al.* 1993). The uptake of glucose into the cell by the phosphoenolpyruvate-dependent glucose phosphotransferase system (PTS) would decrease the level of phosphorylation of one of the PTS components,

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Summary Figure Mechanism of glucose–lactose diauxie in *E. coli.*

enzyme IIA^{Glc}. The resulting dephosporylated enzyme IIA^{Glc} is believed to prevent the uptake of the inducer by binding to the *lac* permease (Osumi & Saier Jr 1982; Nelson *et al.* 1983).

When both glucose and lactose are present, *Escherichia coli* cells preferentially utilize glucose and the use of lactose is prevented until the glucose is depleted, resulting in diauxic growth (diauxie) (Monod 1947; Epstein *et al.* 1966). The diauxie is a typical example of glucose effect. One famous model states that glucose inhibits *lac* expression by reducing the level of cAMP (see Fig. 1). This model is clearly an oversimplification, because it is apparent that inducer exclusion is involved in the glucose–lactose diauxie (reviewed by Magasanik 1970; Saier Jr 1989; Roseman & Meadow 1990; Postma *et al.* 1993). It should be noted that the ability of glucose to reduce the internal concentration of the inducer had been shown to play an important role in glucose–lactose diauxie before the role of cAMP was discovered (Loomis & Magasanik 1967). However, cAMP is still believed to be at least one of the important mediators of glucose repression in the glucose–lactose system. Thus, one common view among researchers is that glucose represses the expression of the *lac* operon by lowering the intracellular concentrations of both cAMP and the pre-inducer, lactose (reviewed by Magasanik & Neidhardt 1987; Saier Jr 1989; Roseman & Meadow 1990; Postma *et al.* 1993).

Surprisingly, the way in which the cAMP level fluctuates during glucose–lactose diauxie has not yet been focused on. In other words, there is no direct evidence that a reduction of cAMP level is indeed involved in the glucose effect. In this study, we examined the roles of cAMP and CRP in glucose–lactose diauxie by determining the levels of these two factors. We found that a reduction of the CRP-cAMP level is not responsible for glucose repression and that glucose inhibits the expression of the *lac* operon, mainly by lowering the intracellular inducer concentration.

Results

Effect of carbon sources on cAMP and CRP levels

We determined total cAMP levels and β-galactosidase

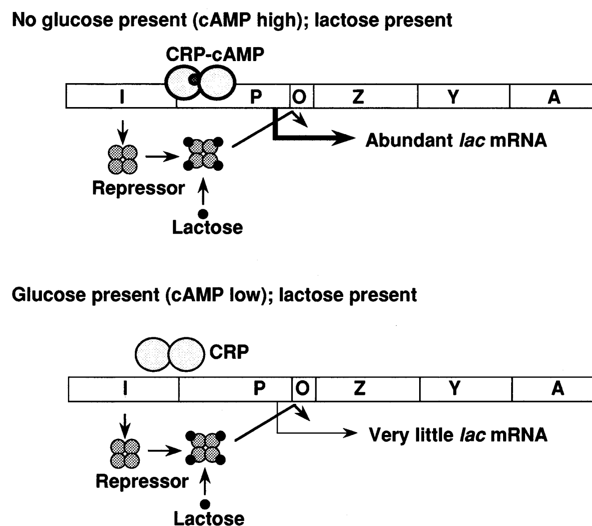

Figure 1 Classical model for glucose effect on *lac* operon expression in glucose–lactose system. Glucose and lactose levels control the initiation of transcription of the *lac* operon through their effects on the *lac* repressor and CRP. When lactose is present and glucose is absent (upper panel), the *lac* operon is effectively transcribed. This is because the presence of lactose inactivates the *lac* repressor, and the absence of glucose causes an increase in cAMP, which activates CRP. When both lactose and glucose are present (lower panel), very little *lac* mRNA is transcribed because the presence of glucose reduces the cAMP level thereby inactivating CRP. (Modified from Darnell *et al.* 1995)

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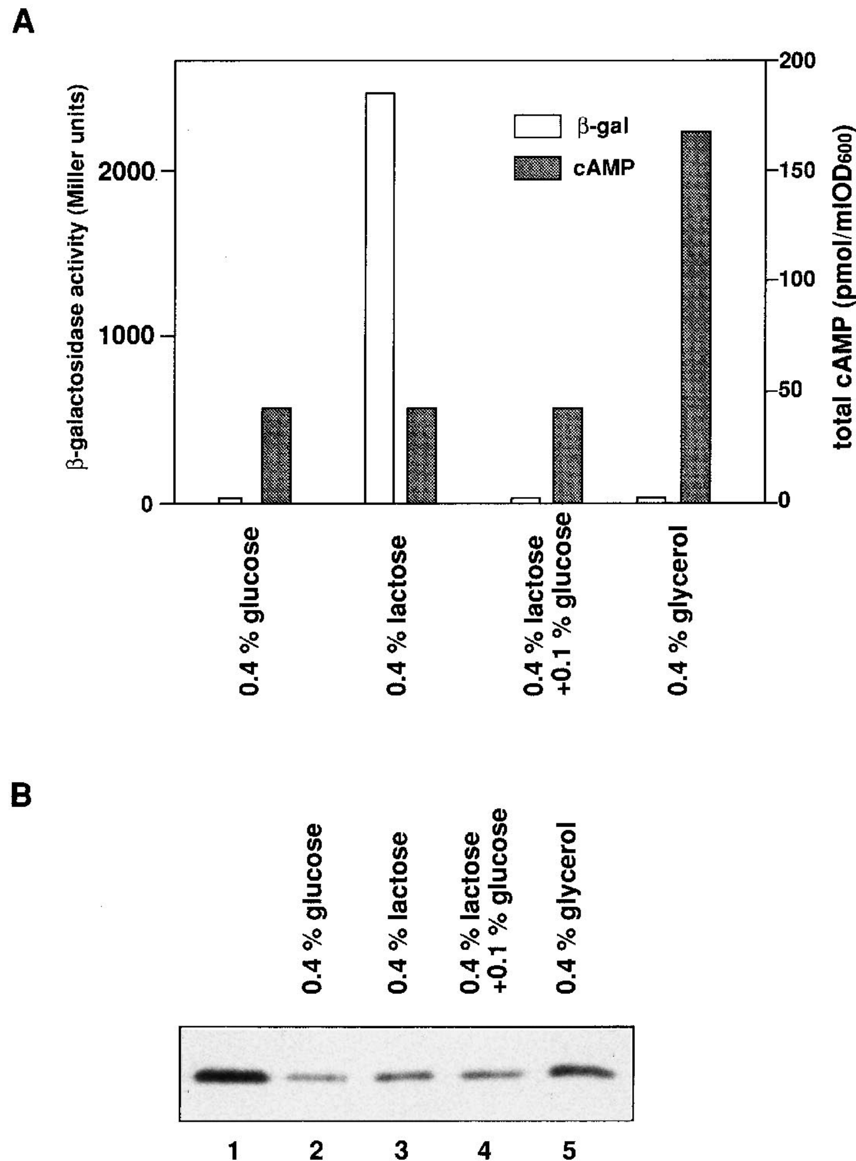

Figure 2 Effects of carbon sources on the levels of cAMP, CRP and β-galactosidase. (A) Total cAMP level and β-galactosidase activity. W3110 cells were grown in M9 medium containing various carbon sources. Culture samples were taken at an OD_{600} of 0.6, and total cAMP level and β-galactosidase activity were determined. Each bar is an average value obtained from three independent experiments. (B) CRP level. Cell extracts equivalent to total $OD_{600} = 0.01$ were analysed by Western blotting (lanes 2–5). Lane 1 corresponds to 5 ng of purified CRP.

activities in cells that were exponentially growing on different carbon sources. The cAMP level was approximately four times higher in glycerol-grown cells than in glucose-grown cells (Fig. 2A) as has been reported previously (Buettner *et al.* 1973; Wayne & Rosen 1974; Epstein *et al.* 1975). To our surprise, the cAMP level in lactose-grown cells was essentially the same as that in glucose-grown cells. We also determined CRP levels in cells growing on glucose and lactose. As shown in Fig. 2B, little difference in CRP level was observed. Then, we examined whether or not the addition of glucose to lactose medium lowers cAMP levels. As shown in Fig. 2A, the presence of 0.1% glucose did not affect the cAMP level while it caused a strong inhibition of β-galactosidase synthesis. These observations indicate that the strong repression of β-galactosidase activity by

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glucose is not due to a reduction of the cAMP level. In addition, the intracellular CRP level in the lactose medium was little affected by the addition of glucose (Fig. 2B).

Levels of cAMP and CRP during glucose–lactose diauxie

The effect of glucose on the cAMP level was further examined by monitoring changes in cAMP levels during diauxic growth in a glucose–lactose medium. The expression of the *lac* operon was strongly repressed during the first exponential phase. β-galactosidase synthesis begins during the lag phase and dramatically increases when the cells enter the second exponential phase (Fig. 3A). The intracellular cAMP level was about 4 μM and did not change significantly during the first exponential phase. After the depletion of glucose, the cAMP level rose sharply to 15 μM and decreased rapidly up to 2 μM when cells entered the second exponential phase (Fig. 3B). We also determined the CRP levels at various points throughout diauxic growth (Fig. 3C). The levels of CRP increased slightly when cells entered the lag phase. However, the difference in CRP levels between the glucose- and lactose-grown phases was marginal. These data strongly suggest that glucose repression of the *lac* operon in the glucose–lactose system is not due to a decrease in the CRP-cAMP level.

Glucose repression is independent of cAMP

Ullmann & Monod (1968) reported that exogenous cAMP eliminated the lag phase of cell growth in a glucose–lactose mixture. In fact, diauxic growth was suppressed by the addition of 5 mM cAMP to the glucose–lactose medium (Fig. 4). However, under these conditions, a significant inhibition of β-galactosidase expression was observed during the early log phase. This result is again in conflict with the view that cAMP mediates the glucose effect in the glucose–lactose system.

If glucose repression in the glucose–lactose system is not mediated by cAMP, it would occur in cells lacking cAMP. To test this possibility, we monitored cell growth and β-galactosidase activity in the strain IT1203 (W3110 Δ*cya*::Kan *crp*B1*). This strain produces no detectable cAMP due to a deletion of the *cya* gene. In addition, it carries mutations in the *crp* gene, resulting in a cAMP independent CRP (CRP*) which activates the *lac* operon in the absence of cAMP (Tagami

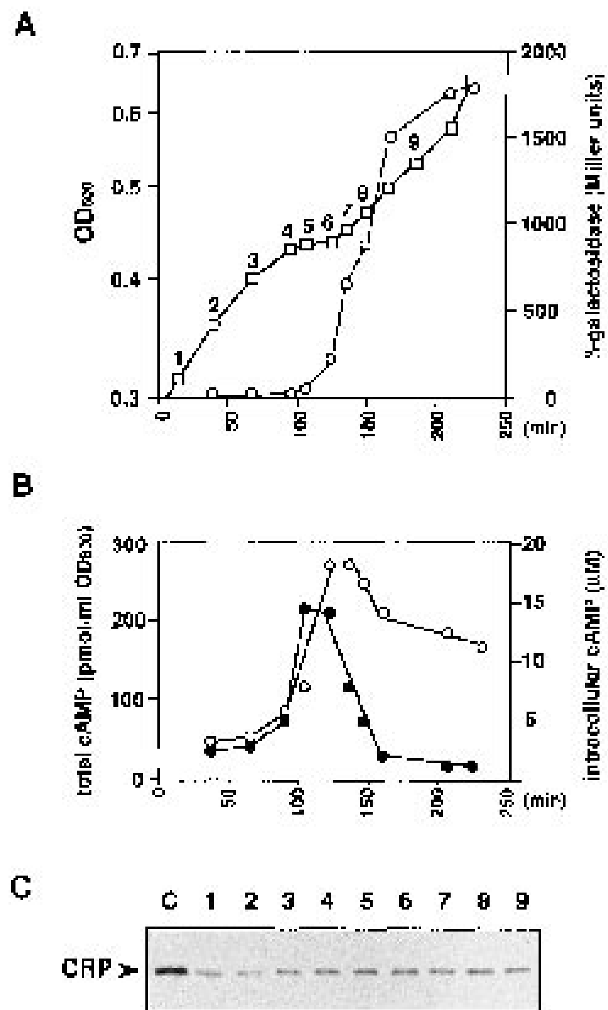

Figure 3 Changes in the levels of β-galactosidase, cAMP, and CRP during glucose–lactose diauxic growth. (A) W3110 cells were grown in M9 medium containing 0.04% glucose and 0.2% lactose. Optical density (□) and β-galactosidase activity (○) were determined at frequent intervals. (B) At points indicated by numbers in (A), total (○) and intracellular (●) cAMP levels were determined. (C) At points indicated by numbers in (A), CRP levels were determined by Western blotting. Cell extracts equivalent to total $OD_{600} = 0.01$ were analysed (lanes 1–9). Lane C corresponds to 5 ng of purified CRP.

et al. 1995). The results are shown in Fig. 5A. Typical diauxic growth and a strong repression of β-galactosidase activity by glucose were observed. Thus, glucose repression and diauxic growth occurred without cAMP. The levels of CRP* protein did not vary significantly during diauxic growth (Fig. 5B), indicating that the control of CRP* level is also not involved in glucose repression.

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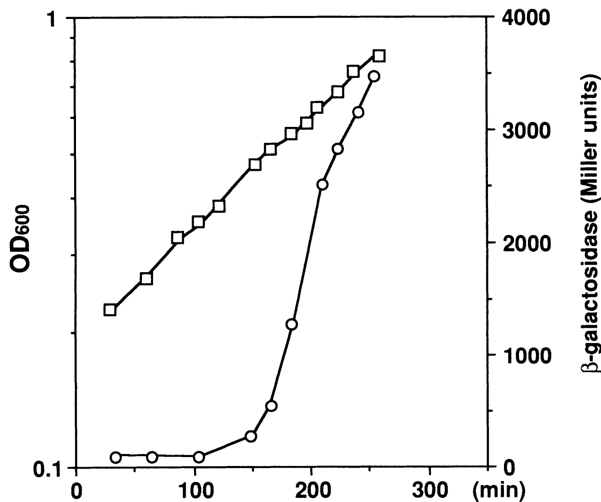

Figure 4 Effect of exogenous cAMP on diauxic growth and β-galactosidase activity. W3110 cells were grown on M9 medium containing 0.04% glucose and 0.2% lactose in the presence of 5 mM cAMP. Samples were removed for optical density (□) and β-galactosidase activity (○) determination.

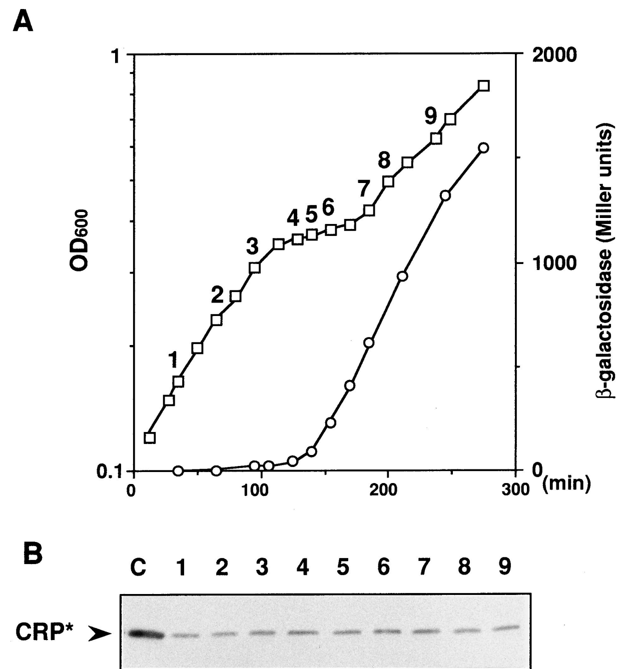

Figure 5 Growth curve, β-galactosidase activity, and CRP* levels of Δ*cya crp** cells growing on glucose–lactose mixture. IT1203 (W3110 Δ*cya*::Kan *crp*B1*) cells were grown in M9 medium containing 0.04% glucose and 0.2% lactose. (A) At indicated time, samples were removed for optical density (□) and β-galactosidase activity (○) determinations. (B) At points indicated by numbers in (A), CRP levels were determined by Western blotting. Cell extracts equivalent to total $OD_{600} = 0.01$ were analysed (lanes 1–9). Lane C corresponds to 5 ng of purified CRP.

Inactivation of the *lac* repressor eliminates glucose repression

If glucose repression in glucose–lactose medium were solely due to the inducer exclusion mechanism, glucose would no longer repress the expression of β-galactosidase when the *lac* repressor is absent. To test this, we monitored the expression of β-galactosidase in a glucose–lactose medium using a strain in which the *lacI* gene was disrupted by Tn*10*. As shown in Fig. 6A, inactivation of the *lacI* gene abolished both the inhibition of β-galactosidase expression and diauxic growth. We also measured β-galactosidase activity in wild-type cells growing on a glucose–lactose medium supplemented with a large excess of IPTG in which the *lac* repressor is assumed to be inactivated. The glucose effect on the *lac* operon and diauxie were again abolished (Fig. 6B). The results clearly indicate that glucose exerts its effect simply by modulating the activity of the *lac* repressor in glucose–lactose medium. It is known that the nonphosphorylated form of enzyme IIA^{Glc} inhibits *lac* permease and the uptake of glucose increases the level of the nonphosphorylated form of enzyme IIA^{Glc} (reviewed by Postma *et al.* 1993). Therefore, the disruption of the *crr* gene coding for IIA^{Glc} should eliminate glucose repression. In fact, glucose repression was no longer observed in the crr^- cells (data not shown).

Discussion

New model for the glucose effect in glucose–lactose diauxie

Expression of the *lac* operon was almost completely repressed during the first exponential phase of glucose–lactose diauxie. In the present work, we focused on the extent to which the regulation of cAMP, CRP, and the *lac* repressor contribute to glucose repression in the glucose–lactose system. We found that the cAMP level in cells growing on lactose was not higher than that in cells growing on glucose. Furthermore, the addition of exogenous cAMP did not abolish strong glucose repression during the glucose-grown phase. The fact that glucose repression occurs even in *crp** Δ*cya* cells showed that the modulation of the cAMP level is not essential for glucose repression. In addition, the little difference in CRP levels between the glucose- and lactose-grown phases shows that the regulation of CRP

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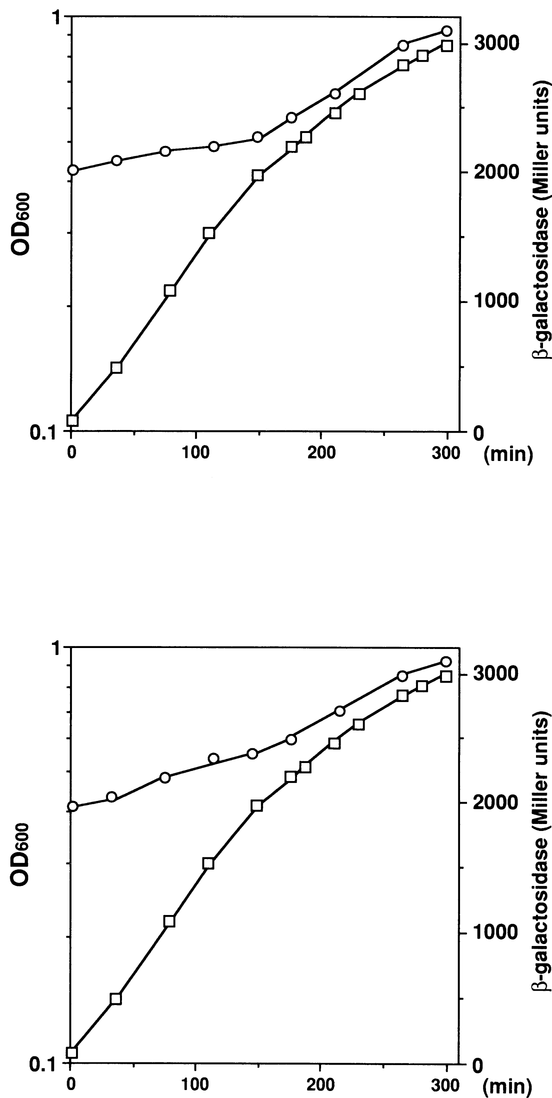

Figure 6 Effect of inactivation of *lacI* gene on glucose effect. (A) IT1169 (W3110 *lacI*::Tn*10*) cells were grown in M9 medium containing 0.04% glucose and 0.2% lactose. At indicated time, samples were removed for optical density (□) and β-galactosidase activity (○) determination. (B) W3110 cells were grown on M9 medium containing 0.04% glucose and 0.2% lactose in the presence of 0.5 mM IPTG. At indicated time, samples were removed for determination of optical density (□) and β-galactosidase activity (○).

also plays no role in glucose repression. We concluded that the positive factor, CRP-cAMP, is not a mediator in glucose repression in the glucose–lactose system. Finally, the inactivation of the *lac* repressor, either through the addition of a large amount of IPTG or the disruption of the *lacI* gene eliminated both glucose repression and diauxic growth. This suggests that glucose repression in the glucose–lactose diauxie is totally attributed to an elevated concentration of active *lac* repressor. Based on these observations we propose a new model for the glucose effect in the glucose–lactose system (Fig. 7). When cells find lactose alone, the sugar is transported through *lac* permease which is present at a basal level. The transported lactose is converted to a natural inducer, allolactose (Burstein *et al.* 1965) and releases the repression of the *lac* operon. When both glucose and lactose are present in the growth medium, the uptake of lactose is strongly inhibited by glucose because of an increase in the nonphosphorylated form of IIA^{Glc}, an inhibitor of *lac* permease.

Diauxic growth and effect of exogenous cAMP

While the addition of exogenous cAMP did not eliminate glucose repression of the *lac* operon, it abolished diauxic growth. We interpret this as follows. In the absence of exogenous cAMP, cells start to take up lactose by using a basal level of permease after the depletion of glucose. Then the expression of the *lac* operon gradually increases, resulting in the synthesis of more permease. However, since the level of permease before the depletion of glucose is extremely low, a significant amount of time is required for the synthesis of enough permease to allow the efficient induction of the *lac* operon. When exogenous cAMP is added, the basal level of *lac* expression is elevated several fold because of an increase in the concentration of CRP-cAMP. The increase in the basal level of *lac* permease should support cells to take up lactose efficiently when glucose is depleted. This uptake of lactose is presumably sufficient to eliminate the lag phase between the glucose- and lactose-grown phases.

Role of CRP-cAMP in catabolite repression

The permanent effect of glucose, which is independent from the repressor-mediated negative regulation, has often been referred to as catabolite repression (Pastan & Perlman 1970; Magasanik 1970). How are the present results to be reconciled with the role of CRP-cAMP as a mediator of catabolite repression? Catabolite repression has usually been studied by using cells induced by IPTG on glycerol or succinate medium (Perlman *et al.* 1969; Pastan & Perlman 1970; Ishizuka *et al.* 1993). In the absence of glucose, CRP-cAMP levels are high enough to fully activate the *lac* operon in these media. It should be noted that CRP-cAMP levels in lactose or glucose media are not sufficient to produce maximum expres-

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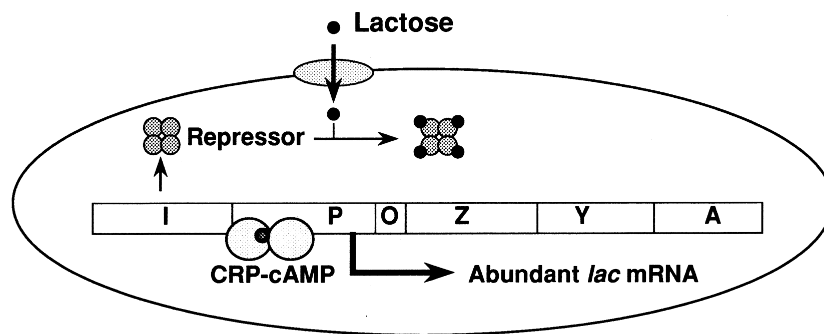

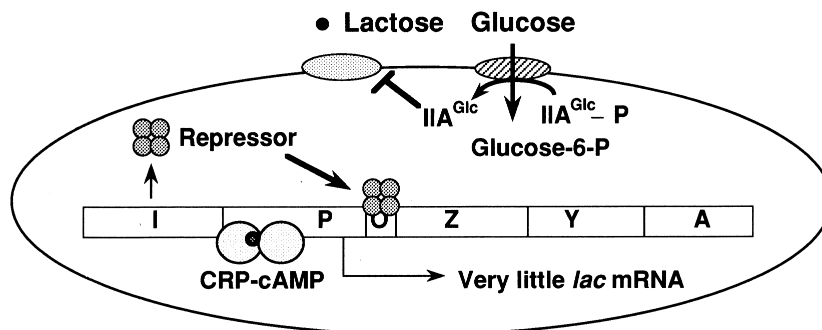

Figure 7 New model for glucose effect on *lac* operon expression in the glucose–lactose system. When lactose is present and glucose is absent (upper panel), lactose is incorporated into cells through lactose permease (shown as shaded oval) and the *lac* repressor is inactivated. The cAMP-CRP level is moderate to allow efficient, although not maximal, transcription of the *lac* operon. When both lactose and glucose are present (lower panel), very little *lac* mRNA is transcribed. This is because the incorporation of glucose through the $IICB^{Glc}$ protein (shown as hatched oval) causes the accumulation of the nonphosphorylated form of IIA^{Glc} which inhibits lactose permease. Thus, the concentration of the inducer is low and the *lac* repressor is active to repress transcription of the *lac* operon. The presence of glucose does not affect the levels of cAMP and CRP.

sion of the *lac* operon. The addition of glucose to lactose medium does not affect both the cAMP and CRP levels. On the other hand, glucose reduces both cAMP and CRP levels when added to cells growing on a poor carbon source such as glycerol or succinate, causing catabolite repression of the *lac* operon. It is interesting to note that lactose causes catabolite repression when added to cells growing on glycerol medium as glucose does (unpublished results). Thus, whereas glucose lowers cAMP level and inhibits lactose permease, lactose lowers cAMP without inhibiting its own entry.

Role of cAMP regulation in glucose–lactose diauxie

While the role of cAMP-CRP as a pleiotropic transcription factor is well established (reviewed by Botsford & Harman 1992; Kolb *et al.* 1993), the present study suggests a novel role for the regulation of cAMP level in cell physiology. The intracellular cAMP level increases transiently during the lag phase in diauxic growth. This increase in the cAMP level stimulates *crp* expression through its positive autoregulatory circuit. As a result, the depletion of glucose significantly increases intracellular concentration of the CRP-cAMP complex. The increase in CRP-cAMP level should minimize lag time before lactose utilization. Once cells start to metabolize lactose efficiently, intracellular cAMP again falls quickly. The transient increase in cAMP levels after depletion of glucose may correspond to an early observation that the intracellular concentration of cAMP rose drastically as glucose disappeared from the medium (Makman & Sutherland 1965; Buettner *et al.*

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1973). How cells regulate the intracellular cAMP level in such a dynamic fashion remains an important unanswered question.

Experimental procedures

Bacterial strains, medium and growth conditions

The wild-type *E. coli* strain used in this study was W3110. IT1169 (W3110 *lacI*::Tn*10*) was constructed from W3110 by P1 transduction using IT1002 (*lacI*::Tn*10*, Tagami *et al.* 1995) as a donor. IT1203 (W3110 Δ*cya*::Kan *crp* B1*) was constructed from IT1106 (W3110 *crp* B1*) by P1 transduction using HT26 (W3110 Δ*cya*::Kan) as a donor. The *crp* B1* allele has two amino acid substitutions (T28K and A144T) which have been described elsewhere (Tagami *et al.* 1995). HT26 (W3110 Δ*cya*::Kan), in which the *cya* gene is deleted, was constructed through linear transformation as described elsewhere (in preparation). The replacement of the *cya* gene with a kanamycine resistance gene (Kan) in HT26 was confirmed by southern hybridization. M9 medium was supplemented with 0.001% thiamine. In most experiments, an overnight culture of cells grown in LB medium was diluted in M9 media containing various carbon sources and grown aerobically at 37 °C. The bacterial growth was monitored by determining the optical density at 600 nm (OD_{600}).

Immunoblot analysis of CRP

Culture samples equivalent to 1.0 OD_{600} were centrifuged and the residual materials were suspended in 500 μL of H_2O. To solubilize cells, the cell suspensions were mixed with 500 μL of 2 × loading buffer (4% SDS, 10% 2-mercaptoethanol, 125 mM Tris-HCl, pH 8.0, 20% glycerol, 0.2% bromophenol blue) and heated for 10 min at 100 °C. Total proteins of the indicated amount were loaded onto 0.1% SDS–13% polyacrylamide gels and electrophoresed. After electrophoresis, Western blotting was performed as described previously (Ishizuka *et al.* 1993), using a polyclonal anti-CRP antibody and a horseradish peroxidase-conjugated secondary antibody.

β-galactosidase activity

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

Determination of cAMP level

cAMP was assayed using the cAMP EIA system (Amersham) according to the method specified by the manufacturer. To determine the total cAMP level, culture samples were taken and heated for 5 min, then centrifuged at 12 000 ***g*** for 2 min at 4 °C. The cAMP levels of supernatants were determined as total cAMP levels which were expressed as pmol/OD unit (at 600 nm) of the culture sample. To determine the intracellular cAMP level, culture samples were taken and centrifuged at 12 000 ***g*** for 2 min at 4 °C. The pellets were suspended in 20 μL of H_2O, and heated for 5 min at 100 °C. Samples were centrifuged at 12 000 ***g*** for 2 min at 4 °C, and supernatants were removed and mixed with 60 μL ethanol, then centrifuged at 12 000 ***g*** for 10 min at 4 °C. The supernatants were dried, and pellets were suspended in 10 μL of H_2O and used for the determination of intracellular cAMP levels. The intracellular concentration of cAMP was calculated on the assumption that an OD_{600} of 1.4 corresponds to 10^9 cells/mL (Miller 1972) and the volume of the cells is 2×10^{-12} mL (Joseph *et al.* 1982).

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References

Botsford, J.L. & Harman, J.G. (1992) Cyclic AMP in prokaryotes. *Microbiol. Rev.* **56**, 100–122.
Buettner, M.J., Spitz, E. & Rickenberg, H.V. (1973) Cyclic adenosine 3′:5′-monophosphate in *Escherichia coli*. *J. Bacteriol.* **14**, 1068–1073.
Burstein, C., Cohn, M., Kepes, A. & Modod, J. (1965) Role of lactose and its metabolic products in the induction of the lactose operon in *Escherichia coli*. *Biochim. Biophys. Acta* **95**, 634–639.
Epstein, W., Naono, S. & Gros, F. (1966) Synthesis of enzymes of the lactose operon during diauxic growth of *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **24**, 588–592.
Epstein, W., Rothman-Denes, L.B. & Hesse, J. (1975) Adenosine 3′:5′-cyclic monophosphate as mediator of catabolite repression in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **72**, 2300–2304.
Hanamura, A. & Aiba, H. (1992) A new aspect of transcriptional control of the *Escherichia coli crp* gene: positive autoregulation. *Mol. Microbiol.* **6**, 2489–2497.
Ishizuka, H., Hanamura, A., Inada, T. & Aiba, H. (1994) Mechanism of the down-regulation of cAMP receptor protein by glucose in *Escherichia coli:* role of the autoregulation of the *crp* gene. *EMBO J.* **13**, 3077–3082.
Ishizuka, H., Hanamura, A., Kunimura, T. & Aiba, H. (1993) A lowered concentration of cAMP receptor protein caused by glucose is an important determinant for catabolite repression in *Escherichia coli*. *Mol. Microbiol.* **10**, 341–350.
Joseph, E., Bernsley, C., Guiso, N. & Ullmann, A. (1982) Multiple regulation of the activity of adenylate cyclase in *Escherichia coli*. *Mol. Gen. Genet.*185, 262–268.
Kolb, A., Busby, S., Buc, H., Garges, S. & Adhya, S. (1993) Transcriptional regulation by cAMP and its receptor protein. *Annu. Rev. Biochem.* **62**, 749–95.
Lodish, H., Baltimore, D., Berk, A., Zipursky, S. L., Matgudaira, P. & Darnell, J. (1995) *Molecular Cell Biology.* New York: W.H. Freeman.
Loomis, W.F. & Magasanik, B. (1967) Glucose–lactose diauxie in *Escherichia coli*. *J. Bacteriol.* **93**, 1397–1401.

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Magasanik, B. (1970) Glucose effects: inducer exclusion and repression. In: *The Lactose Operon* (eds J. Beckwith & D. Zipser), pp. 189–220. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Magasanik, B. & Neidhardt, F. (1987) Regulation of carbon and nitrogen utilization. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (ed. F. Neidhardt), pp. 1318–1325. Washington, DC: American Society for Microbiology.

Makman, R.S. & Sutherland, E.W. (1965) Adenosine 3′,5′-phosphate in *Escherichia coli. J. Biol. Chem.* **240**, 1309–1314.

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

Monod, J. (1947) The phenomenon of enzymatic adaptation. *Growth* **11**, 223–289.

Nelson, S.O., Wright, J.K. & Postma, P.W. (1983) The mechanism of inducer exclusion. Direct interaction between purified III^{Glc} of the phosphoenolpyruvate: sugar phosphotransferase system and the lactose carrier of *Escherichia coli. EMBO J.* **2**, 715–720.

Osumi, T. & Saier, M.H. Jr (1982) Regulation of lactose permease activity by the phosphoenolpyruvate: sugar phosphotransferase system; evidence for direct binding of the glucose-specific enzyme III to the lactose permease. *Proc. Natl Acad. Sci. USA* **79**, 1457–1461.

Pastan, I. & Adhya, S. (1976) Cyclic adenosine 5′-monophosphate in *Escherichia coli. Bacteriol. Rev.* **40**, 527–551.

Pastan, I. & Perlman, R. (1970) Cyclic adenosine monophosphate in bacteria. *Science* **169**, 339–344.

Perlman, R.L., de Crombrugghe, B. & Pastan, I. (1969) Cyclic AMP regulates catabolite and transient repression in *E. coli. Nature* **223**, 810–812.

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

Reznikoff, W.S. (1992) The lactose operon-controlling elements: a complex paradigm. *Mol. Microbiol.* **6**, 2419–2422.

Reznikoff, W.S. & Abelson, J.N. (1978) The *lac* promoter. In: *The Operon* (eds J.H. Miller & W.S. Reznikoff), pp. 221–243. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Roseman, S. & Meadow, N.D. (1990) Signal transduction by the bacterial phosphotransferase system. *J. Biol. Chem.* **265**, 2993–2996.

Saier, M.H. Jr, (1989) Protein phosphorylation and allosteric control of inducer exclusion and catabolite repression by the bacterial phosphoenolpyruvate: sugar phosphotransferase system. *Microbiol. Rev.* **53**, 109–120.

Tagami, H., Inada, T., Kunimura, T. & Aiba, H. (1995) Glucose lowers CRP* levels resulting in repression of the *lac* operon in cells lacking cAMP. *Mol. Microbiol.* **17**, 251–258.

Ullmann, A. & Monod, J. (1968) Cyclic AMP as an antogonist of catabolite repression in *Escherichia coli. FEBS Lett.* **2**, 57–60.

Ullmann, A. & Danchin, A. (1983) Role of cyclic AMP in bacteria. *Adv. Cyclic Nucleotide Res.*15, 1–53.

Wayne, P.K. & Rosen, O.M. (1974) Cyclic 3′:5′-adenosine monophosphate in *Escherichia coli* during transient and catabolite repression. *Proc. Natl Acad. Sci. USA* **71**, 1436–1440.

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