Mechanism of catabolite repression in the *bgl* operon of *Escherichia coli*: involvement of the anti-terminator BglG, CRP-cAMP and EIIA^{Glc} in mediating glucose effect downstream of transcription initiation

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Abstract

***Background*: Expression of the *bgl* operon of *Escherichia coli*, involved in the regulated uptake and utilization of aromatic β-glucosides, is extremely sensitive to the presence of glucose in the growth medium. We have analysed the mechanism by which glucose exerts its inhibitory effect on *bgl* expression.**

***Results*: Our studies show that initiation of transcription from the *bgl* promoter is only marginally sensitive to glucose. Instead, glucose exerts a more significant inhibition on the elongation of transcription beyond the rho-independent terminator present within the leader sequence. Transcriptional analyses using plasmids that carry mutations in *bglG* or within the terminator, suggest that the target for glucose-mediated repression is the anti-terminator protein, BglG. Introduction of multiple copies of *bglG* or the presence of mutations that inhibit its phosphorylation by Enzyme II^{Bgl} (BglF), result in loss of glucose repression. Studies using *crp*, *cya* and *crr* strains show that both CRP-cAMP and the Enzyme IIA^{Glc} (EIIA^{Glc}) are involved in the regulation. Although transcription initiation is normal in a *crp*, *cya* double mutant, no detectable transcription is seen downstream of the terminator, which is restored by a mutation within the terminator. Transcription past the terminator is also partly restored by the addition of exogenous cAMP to glucose-grown cultures of a *crp*$^{+}$ strain. Glucose repression is lost in the *crr* mutant strain.**

***Conclusions*: The results summarized above indicate that glucose repression in the *bgl* operon is mediated at the level of transcription anti-termination, and glucose affects the activity of BglG by altering its phosphorylation by BglF. The CRP-cAMP complex is also involved in this regulation. The results using the *crr* mutant suggest a negative role for EIIA^{Glc} in the catabolite repression of the *bgl* genes.**

Introduction

The mechanisms by which bacteria mediate carbon catabolite repression (CCR) and alter gene expression, in response to changes in the nutritional availability, have been extensively examined in both Gram-positive and Gram-negative bacteria (reviewed in Wanner *et al.* 1978; Saier 1989; Saier *et al.* 1996). Although the regulatory processes manifest themselves in a functionally similar manner, there are clear mechanistic differences in these two classes of eubacteria. Both require the phosphoenolpyruvate-dependent phosphotransferase system (PTS) for exerting CCR (reviewed by Postma *et al.* 1993 and Saier & Reizer 1994). However, the target and the mediator proteins, as well as the mechanism by which the signal for catabolite repression is transmitted, are significantly different.

In Gram-positive bacteria, CCR is triggered by a metabolite-activated ATP-dependent protein kinase, which phosphorylates the general PTS protein HPr at a critical serine residue (Deutscher & Saier 1983). This seryl-phosphorylated-HPr causes the stimulation of sugar-P release from the cytoplasmic compartment of the cell (inducer expulsion) and restricts the entry of sugars into the cell (inducer exclusion) regardless of whether the uptake occurs via the PTS or non-PTS

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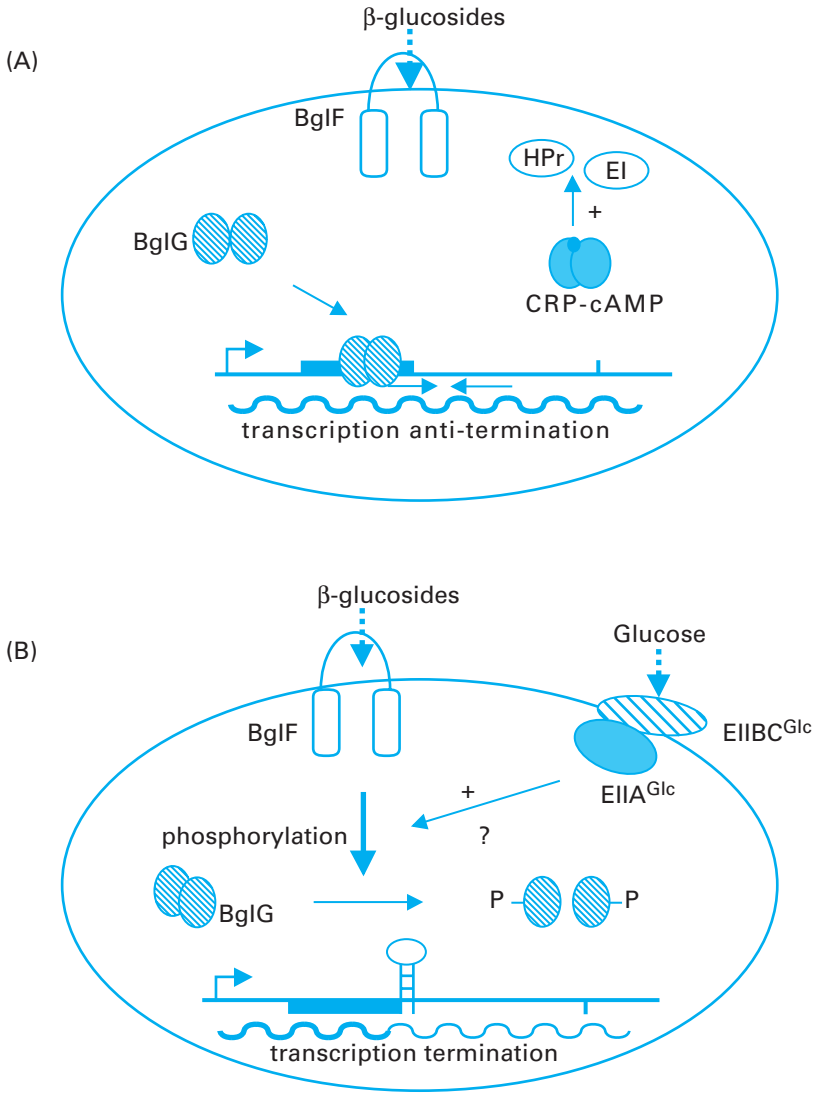

Summary Figure A model for the mechanism of catabolite repression in the *bgl* operon of *E. coli*. (A) When cells are grown in the absence of glucose, presence of β-glucosides in the medium results in the dephosphorylation of the anti-terminator BglG, leading to its activation. The active BglG dimer mediates anti-termination of transcription resulting in expression of downstream genes. This is facilitated by the indirect action of the CRP-cAMP complex, which is known to be involved in the expression of the PTS components. (B) When cells are grown in presence of glucose and inducing amounts of β-glucosides, the BglF-mediated phosphorylation of the anti-terminator BglG is enhanced, which is further stimulated by $EIIA^{Glc}$. Phosphorylation of BglG leads to its inactivation causing a drastic decrease in transcription read-through beyond the *bgl* leader sequence although transcription initiates normally. The precise mechanism by which CRP-cAMP and $EIIA^{Glc}$ mediate their effect remains to be established.

permeases (Ye *et al.* 1994). It also activates the transcription factor CcpA, which causes the repression of catabolic operons (Deutscher *et al.* 1994, 1995). Thus, HPr acts as the key player in mediating the CCR of both PTS and non-PTS sugars.

In Gram-negative bacteria such as *Escherichia coli*, the key mediator of catabolite repression is the enzyme IIA^{Glc} ($EIIA^{Glc}$), another cytosolic member of the PTS. Transport of a readily utilisable sugar, such as glucose, causes the dephosphorylation of $EIIA^{Glc}$. Unphosphorylated $EIIA^{Glc}$ leads to inducer exclusion by blocking the entry of a number of non-PTS sugars (e.g. lactose, maltose, glycerol, melibiose, etc.) by allosterically inhibiting their respective permeases (reviewed by Postma *et al.* 1993). It also leads to a decrease in the intracellular levels of cAMP and the cAMP Receptor Protein (CRP), which are required for the optimal expression of a number of catabolic operons (Epstein *et al.* 1975; Ishizuka *et al.* 1993; reviewed by Botsford & Harman 1992; Kolb *et al.* 1993).

Although considerable information about catabolite repression is available in both groups of organisms, in the case of *E. coli*, little is known as to how the uptake and metabolism of one PTS sugar is affected by the presence of another more easily utilisable PTS carbon source. We have attempted to examine this by analysing the effect of glucose on the expression of the *bgl* operon of *E. coli*, involved in the catabolism of aromatic β-glucosides such as salicin and arbutin, which require the PTS for their transport (Fox & Wilson 1968; Prasad & Schaefler 1974).

The *bgl* operon encodes a transcriptional regulator,

BglG; a phosphoenolpyruvate-dependent β-glucoside permease EII^{Bgl} (BglF), and a phospho-β-glucosidase, BglB (Prasad & Schaefler 1974; Mahadevan *et al.* 1987; Schnetz *et al.* 1987). Expression of the operon is subject to induction by β-glucosides (Schaefler 1967). This regulation is brought about by BglG and BglF (Mahadevan *et al.* 1987). Transcription from the *bgl* promoter initiates constitutively. In the absence of an inducer, most of the transcripts terminate at a rho-independent terminator within the first 130 nucleotides (Mahadevan & Wright 1987; Schnetz & Rak 1988). Induction of the operon by β-glucosides involves anti-termination of transcription mediated by BglG, a sequence-specific RNA binding protein that interacts with the *bgl* mRNA (Houman *et al.* 1990). When β-glucosides are absent, BglG is phosphorylated by BglF, resulting in its inactivation, causing premature termination of transcription and decreased expression of the downstream *bgl* genes (Amster-Choder *et al.* 1989; Amster-Choder & Wright 1990, 1992; Schnetz & Rak 1990; Chen *et al.* 1997).

The *bgl* operon also shows a strong glucose effect. Earlier studies using reporter gene constructs have shown that, in the presence of glucose, expression of the operon decreases dramatically (Lopilato & Wright 1990). In the current report we have investigated the molecular mechanisms underlying glucose effect in the *bgl* operon. We present evidence that glucose brings about repression by affecting the transcription termination step by modulating the activity of BglG. Since $EIIA^{Glc}$ and CRP-cAMP are the key mediators of catabolite repression for several non-PTS sugars, their role in bringing about the glucose effect in the *bgl* operon was examined. The data presented here show that both $EIIA^{Glc}$ and CRP-cAMP play an important role in *bgl* expression, but that their mode of action is different from other known systems.

Results

Glucose exerts its inhibitory effect on *bgl* expression at steps subsequent to transcription initiation

Earlier studies using *bgl-lac* fusions have shown that expression of the *bgl* operon is extremely sensitive to catabolite repression (Lopilato & Wright 1990). We examined the level of glucose repression in the strain MA200, which carries an insertionally activated *bgl* operon (Mahadevan *et al.* 1987). This strain also harbours a fusion of the *bgl* promoter and terminator to the *lac* genes, present on a lambda prophage. MA200 was grown in minimal medium supplemented with either 0.4% glucose or 0.4% succinate as the carbon source. Salicin at a concentration of 7 mM was added as the inducer. Measurement of β-galactosidase activity from cells grown in the presence or absence of glucose showed that glucose-mediated repression was over 30-fold (Table 1).

To further examine the level at which glucose exerts its inhibitory effect, transcription from the *bgl* promoter in the presence and absence of glucose was quantified using an S1 nuclease protection assay. The *E. coli* K-12 strain, JF201, deleted for the chromosomal copy of the *bgl* operon, was transformed with the plasmid pMN22AE harbouring an insertionally activated *bgl* operon (Singh *et al.* 1995). Total RNA was isolated from cells grown in the presence or absence of glucose (growth conditions same as those for the β-galactosidase assay). Initiation of transcription from the *bgl* promoter was detected using a large excess of an oligonucleotide probe (Probe 1) complementary to the first 42 nucleotides of the *bgl* message. The *bgl* transcripts were normalized to the *amp* transcripts using a probe complementary to the β-lactamase mRNA. The transcriptional analysis revealed several interesting features. Growth in glucose led to a less than twofold decrease in *bgl* transcription compared to that in the succinate–grown cultures (Fig. 1B). When a similar analysis was carried out using a second probe (Probe 2) which is complementary to the coding region (+454 to +496) of first gene of the operon, *bglG*, a sevenfold decrease in transcription was observed in the presence of glucose (Fig. 1C). This indicates that, unlike in the *lac* operon, glucose repression in the *bgl* operon is not exerted at transcription initiation but at steps subsequent to it. Apparently, growth in glucose does not

Table 1 Effect of glucose and cAMP on expression of the *bgl* operon in different strains. Units of β-galactosidase activity shown are the average of at least three independent experiments. 7 mM salicin was used as the inducer (I) and cAMP was used at a final concentration of 5 mM

Strain used	Glucose +I	Succinate +I	Glucose effect
MA200	2	70	35-fold
MA200-1	14	132	9-fold
MA200-33	16	153	10-fold
Strain used	**Glucose +I**	**Glucose +I +cAMP**	**cAMP effect**
MA200	2	8	4-fold

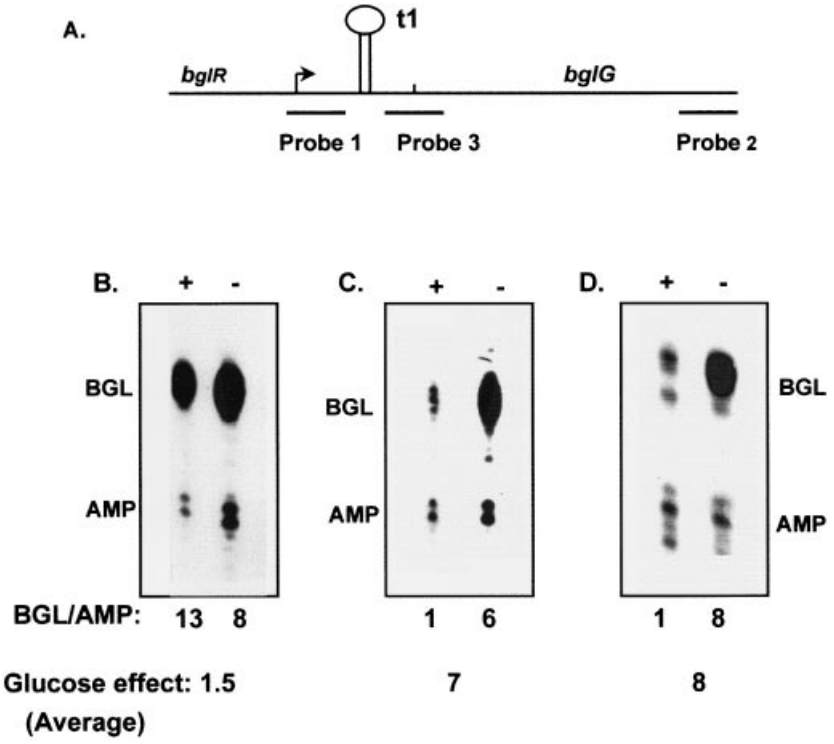

Figure 1 Effect of glucose on expression of the *bgl* operon. (A) The oligonucleotide probes used are shown as dark lines. Arrow indicates the transcription start site and t1 is the terminator preceding *bglG*. Probe 1 corresponds to the transcription initiation region within the regulatory locus, *bglR* (−10 to +42); probe 2 hybridizes within the first gene of the operon (+454 to +496) and probe 3 hybridizes immediately downstream of the terminator (+121 to +161). (B) Transcriptional analysis using RNA isolated from JF201 transformed with pMN22AE using probe 1. '+' and '−' indicate the presence and absence of glucose, respectively. Positions of the *bgl* and *amp* signals are indicated and the ratio of the *bgl* to the *amp* signal corresponding to the specific autoradiogram is presented. Glucose effect is expressed as the ratio of the signal (*bgl*/*amp*) obtained in the succinate grown cultures to the glucose grown cultures. The fold effect indicated in all the figures is the average of at least three independent experiments. (C) Transcription measured using RNA isolated from JF201 transformed with pMN22AE using probe 2. '+' and '−' indicate signals obtained using probe 2 in the presence and absence of glucose, respectively. (D) Transcription from pMN22AE, measured using probe 3, as described above.

affect transcription initiation appreciably, but the *bgl* RNA 500 nucleotides downstream of the start site is drastically decreased.

The target for glucose effect in the *bgl* operon is a transcription terminator

The previous experiment localized the regulatory region for glucose effect to a 500 base pair stretch downstream of the *bgl* promoter. To locate the site of regulation more precisely, a third oligonucleotide probe (Probe 3) complementary to the leader sequence preceding the first gene (+121 to +161) was used for hybridization. Transcriptional analysis using probe 3 (Fig. 1D) showed that glucose caused an eightfold reduction in the *bgl* mRNA, which is comparable with the effect seen using the *bglG* probe (Probe 2). This suggests that the significant glucose effect that is seen further downstream in the previous experiment is a reflection of the regulation occurring within the leader sequence. Probes 1 and 3 hybridize to regions separated by a stretch of only 75 base pairs, which contains the putative target for the inhibitory effect of glucose. Interestingly, this small stretch of RNA contains the rho-independent transcription terminator involved in the regulation of the operon by β-glucosides (Mahadevan & Wright 1987; Schnetz & Rak 1988). The results described above suggest that glucose repression of the *bgl* operon is exerted at the level of transcription termination.

In an attempt to confirm the finding that growth in glucose affects transcription termination, a plasmid harbouring a mutation within the terminator was used in transcription assays. This is a previously characterized

deletion of a single base (at position +100) from a stretch of three consecutive G residues, which results in a reduced efficiency of termination, both *in vivo* and *in vitro* (Mahadevan & Wright 1987). Transcription was analysed in JF201 transformed with the plasmid pANS 5-16, which harbours the mutation in the terminator. Since our earlier experiments have already shown that transcription initiation is insensitive to glucose, only probe 3 was used for this analysis. As shown in Fig. 2A, growth in glucose does not lead to an appreciable decrease in *bgl* expression when the terminator is mutated. This is in contrast to the eightfold decrease seen when the terminator is intact and fully functional. This result confirms that the target for the glucose-mediated repression in the *bgl* operon is the rho-independent transcription terminator and glucose inhibits *bgl* expression by affecting the process of termination.

Glucose interferes with the activity of the anti-terminator, BglG

The experiments described so far indicate that glucose exerts its inhibitory effect on the elongation of transcription by regulating termination. One attractive possibility is that glucose brings about its effect by interfering with the activity of the anti-terminator, BglG. In an attempt to examine this possibility, transcription downstream of the terminator was analysed in the presence of a truncated version of BglG. The plasmid pA8 harbours the intact terminator but has only the first 200 base pairs of *bglG*, which codes for a truncated protein. This protein is not able to function as an anti-terminator as seen by its inability to complement a chromosomal *bglG* mutation. When pA8, transformed in JF201, was used for transcriptional analysis using probe 3, the glucose effect is lost (Fig. 2B), indicating that an intact terminator *per se* is not sufficient to bring about glucose repression. Glucose repression is observed only in the case of BglG-dependent transcription and the BglG-independent low-level basal transcription is unaffected by the presence of glucose.

To further substantiate that glucose requires a functional BglG for mediating its inhibitory effect, we carried out β-galactosidase assays using MA200 transformed with pMN25, a multicopy plasmid carrying the *bglG* gene (Mahadevan *et al.* 1987). MA200 transformed with pBR322 was used as a control, and enzyme assays were carried out in the presence and absence of glucose. The presence of multiple copies of *bglG* in the cells conferred partial relief from the inhibitory effect of glucose (Fig. 3). This implies that glucose interferes with the activity of BglG to cause premature termination of transcription, an effect that can be overcome by increasing the number of functional copies of the anti-terminator.

Glucose affects BglF-mediated phosphorylation of BglG

It has been well documented that the activity of the anti-terminator is dependent upon its phosphorylation status, which in turn modulates its dimeric state

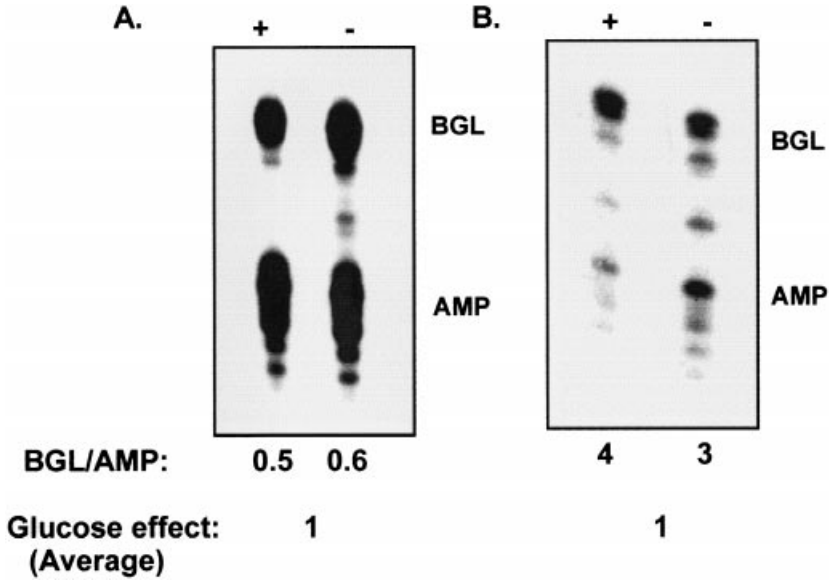

Figure 2 The target for glucose repression is a terminator. (A) Transcription analysis using RNA isolated from JF201 transformed with pANS 5-16 which carries a mutation in the terminator. Only probe 3 has been used for this assay. '+' and '−' indicate the presence and absence of glucose, respectively (B) Transcription analysis using RNA from JF201 transformed with pA8 carrying a truncated copy of *bglG*. Transcription in presence (+) and absence (−) of glucose, respectively, using probe 3.

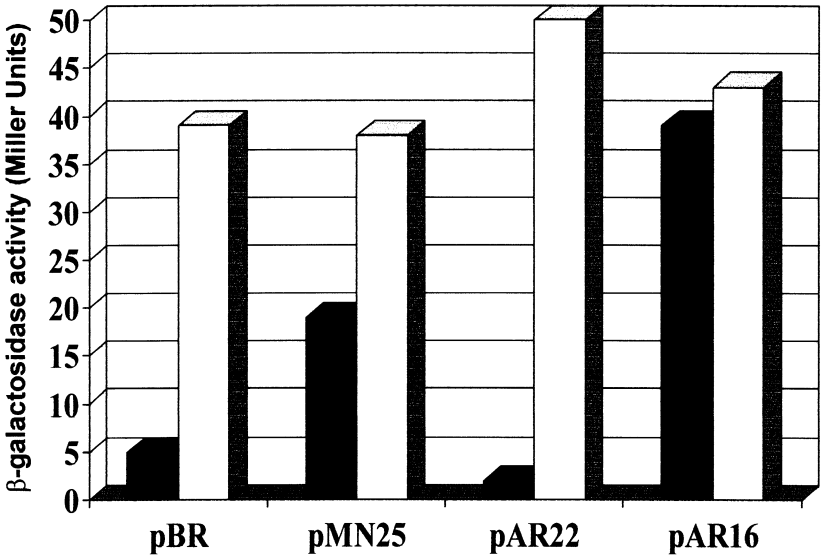

Figure 3 Glucose interferes with BglG activity as an antiterminator by affecting its phosphorylation. Reporter gene expression (β-galactosidase activity) was determined using MA200 transformed with different plasmid constructs. Cells were grown in glucose or succinate and 7 mM salicin was used as the inducer. Filled bars indicate activity in the presence of glucose while empty bars are the units obtained using succinate-grown cultures. Units of β-galactosidase activity plotted are the average of at least three independent experiments.

(Amster-Choder *et al.* 1989; Amster-Choder & Wright 1990, 1992). It is conceivable that growth in glucose affects the phosphorylation status of BglG, thus regulating its activity. To examine this possibility, β-galactosidase assays were carried out with MA200 transformed with pAR16, a plasmid harbouring a mutation in *bglG* (*bglG4*) that renders expression of the operon constitutive and a wild-type copy of the negative regulator, *bglF* (Mahadevan *et al.* 1987). The anti-terminator encoded by the *bglG4* allele has been previously demonstrated to be partially resistant to phosphorylation by BglF (Amster-Choder *et al.* 1989). As a control, the same strain was transformed with pAR22, a plasmid that is similar in structure to pAR16, carrying an activated *bgl* operon harbouring wild-type copies of *bglG* and *bglF*. The results, presented in Fig. 3, show that glucose repression is totally abolished when the phosphorylation of BglG by BglF is compromised by mutation. This observation suggests that in glucose-grown cells, BglF-mediated phosphorylation of BglG leads to its inactivation. This result has been further substantiated by transcriptional analysis carried out using pAR16 transformed in JF201. RNA measurements using an S1 nuclease protection assay showed that, while growth in glucose caused a dramatic decrease in transcription read-through in the wild-type operon, glucose does not affect termination in the BglG4 mutant (data not shown). Similar results were observed when two other mutants, *bglF201* and *bglG33,* were used. MA200-1 carries a mutation in the negative regulator, *bglF*, which renders it inactive, while *bglG33* (harboured in the stain MA200-33) is another previously studied phosphorylation-insensitive mutant of *bglG*. The inhibitory effect of glucose was reduced to less than 10-fold in the mutants as opposed to the greater than 30-fold effect seen in MA200 (Table 1). Thus, glucose modulates the activity of BglG by controlling its phosphorylation by BglF, either by enhancing the phosphorylation or by preventing its dephosphorylation.

CRP-cAMP mediates glucose effect by affecting steps subsequent to transcription initiation

Our previous experiments suggest that transcription initiation from the *bgl* promoter shows only a weak sensitivity to glucose repression, despite the fact that the *bgl* regulatory locus (*bglR*) harbours a strong CRP-cAMP binding site (Reynolds *et al.* 1986). In an attempt to examine whether CRP-cAMP has any role in mediating glucose effect at the downstream site, transcriptional analysis was carried out in the *cya crp* mutant strain, RF5.2, carrying the plasmid pMN22AE. Transformants were grown in glucose or succinate, and *bgl* transcription was analysed as before using the probes 1 and 2. The results of this assay, shown in Fig. 4A, indicate that the levels of *bgl* mRNA measured by probe 1 are comparable in the glucose- and succinate-grown cultures. However, there is a dramatic decrease in transcripts around 500 nucleotides downstream of the transcription start site, as measured by probe 2. This decrease is seen both in the presence and absence of glucose. Therefore, in the absence of CRP-cAMP,

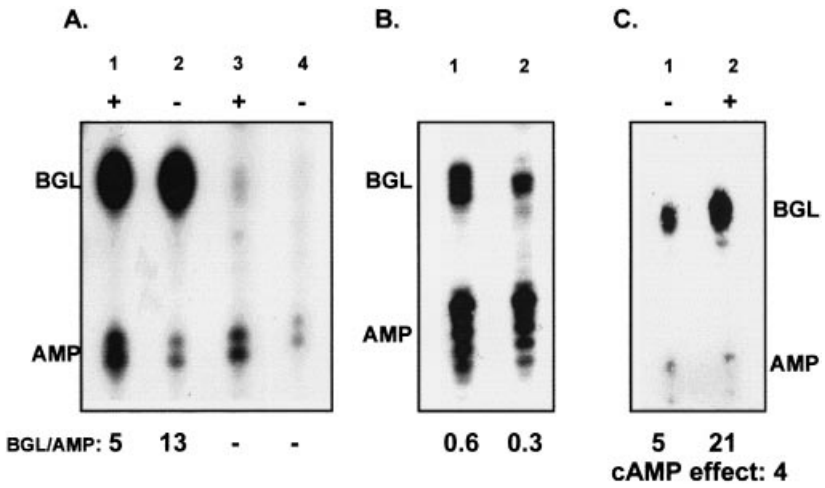

Figure 4 Role of CRP-cAMP in mediating glucose effect. (A) Transcriptional analysis using RNA isolated from RF5.2 (*cya crp*) transformed with pMN22AE in the presence (+) or absence (−) of glucose. Lanes 1 and 2 correspond to signals obtained using probe 1 while lanes 3 and 4 are signals obtained using probe 2. (B) Detection of transcription read-through in a terminator mutant transformed in RF5.2. Transcription was analysed using RNA isolated from RF5.2 transformed with pANS 5-16. Cells were grown in the absence of glucose. Lanes 1 and 2: signals obtained using probes 1 and 3, respectively. Transcription read-through was comparable for cultures grown in the presence of glucose (data not shown) (C) Transcription analysis using RNA isolated from glucose-grown cultures of JF201(*cya*$^+$ *crp*$^+$) transformed with pMN22AE. Probe 2 was used to detect transcription read-through. '+' and '−' indicate the presence or absence of 5 mM cAMP in the growth medium, respectively.

transcription initiation occurs normally, but very few of these transcripts are able to go past the terminator so as to be detected at a downstream site. Consistent with this result, pMN22AE transformed in RF5.2 shows a very weak Bgl$^+$ phenotype on MacConkey medium supplemented with salicin. When the plasmid pANS 5-16, carrying a mutation in the terminator, was introduced in the strain, substantial read-through of transcription could be detected which is 50% of the transcription that is initiated (Fig. 4B). This is in contrast to the situation where the terminator is normal (Fig. 4A). Therefore, the requirement for CRP-cAMP can be overcome when the terminator is mutated. On similar lines, exogenously added cAMP could partly restore downstream transcription in a *crp*$^+$ strain. The strain JF201 (*crp*$^+$ *cya*$^+$) carrying the plasmid pMN22AE was grown in glucose, with or without 5 mM cAMP and transcription was analysed using probe 2. Figure 4C shows that a fourfold larger proportion of transcripts could read-through the leader sequence past the terminator when cAMP was added exogenously. This finding has been substantiated with β-galactosidase assays carried out using the strain MA200. Cells grown in glucose and supplemented with 5 mM cAMP showed a fourfold enhancement in reporter gene expression compared to cells grown in glucose alone (Table 1). Therefore, although glucose repression in the *bgl* operon is not

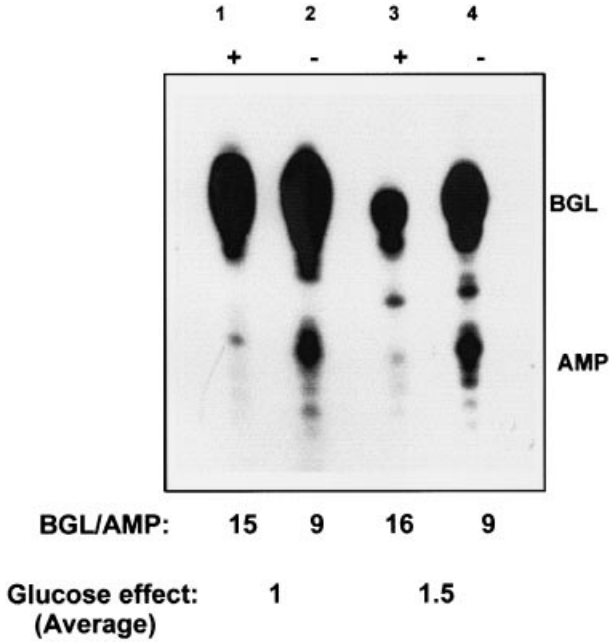

Figure 5 Role of EIIA^{Glc} in mediating glucose effect in the *bgl* operon. Transcription of the *bgl* operon in the *crr* strain IT1199, carrying pMN22AE, was analysed as before. '+' and '−' indicate growth in glucose and succinate, respectively. Lanes 1 and 2 correspond to transcription initiation detected using probe 1, Lanes 3 and 4 are signals corresponding to *bgl* mRNA downstream of the terminator, measured using probe 3.

Table 2 List of bacterial strains and plasmids used in the study

Strain	Description	Source
(A)		
JF201	F$^-$ Δ*lac*X74 Δ(*bgl-pho*)201 *ara thi gyrA*	Reynolds *et al.* (1986)
RF5.2	F$^+$ Δ*lac*U169 Δ*crp*45 Δ*cya*2	A. Wright
IT1199	W3110, Δ*crr*::Kan^R	Kimata *et al.* (1997)
MA200	F$^-$ Δ*lac*X74 *thi bglR 11*(*bglR*::IS*1*) *srl*::Tn*10* *recA56* λ *bglR7 bglG′ lacZ′ lacY* ϕ(*bgl-lac*)	Mahadevan *et al.* (1987)
MA200-1	MA200, *bglF201*	Mahadevan *et al.* (1987)
MA200-33	MA200, *bglG33* (Bgl^C)	Mahadevan *et al.* (1987)
Plasmid	**Genotype and relevant description**	**Reference**
(B)		
pMN22AE	*bglR::IS1 bglG*$^+$ *bglF*$^+$ *bglB*$^+$ *Ap*^{r}	Singh *et al.* (1995)
pMN25	*bglR67 bglG*$^+$ *bglF′ Ap*^{r}	Mahadevan *et al.* (1987)
pAR22	*bglR::IS1 bglG*$^+$ *bglF*$^+$ *bglB*$^+$ *Ap*^{r} *Tc*^{r}	Reynolds *et al.* (1986)
pAR16	*bglR::IS1 bglG4 bglF*$^+$ *bglB*$^+$ *Ap*^{r} *Tc*^{r}	Mahadevan *et al.* (1987)
pA8	*bglR::IS1 bglG′ Ap*^{r}	Moorthy & Mahadevan,unpublished data
pANS 5-16	*bglR::IS1 bglG′ Ap*^{r} (deletion of a single base at +100)	Kharat & Mahadevan, unpublished data

mediated at the step of transcription initiation, the CRP-cAMP complex is essential for expression of the *bgl* genes at steps subsequent to initiation.

Enzyme IIA^{Glc} is required for mediating the glucose effect

One of the primary mechanisms by which glucose repression is brought about in *E. coli* is by the dephosphorylation of enzyme IIA^{Glc}. When glucose is actively being taken up, dephosphorylation of this cytosolic protein causes inhibition of uptake of a number of non-PTS sugars and also leads to a lowering of the intracellular concentrations of CRP-cAMP (reviewed in Saier *et al.* 1996). To examine the role of EIIA^{Glc} in mediating the glucose effect in the *bgl* operon, transcription was analysed in the strain IT1199, which carries a mutation in the *crr* locus encoding the enzyme IIA^{Glc}. Transcription studies were performed using IT1199 transformed with pMN22AE. The results of this analysis are shown in Fig. 5. In the absence of EIIA^{Glc}, growth in glucose did not affect read-through of transcripts past the terminator as indicated by comparable *bgl* signals in the glucose and succinate lanes (detected by probe 3). This indicates that EIIA^{Glc} is necessary for mediating the inhibitory effect of glucose. Transcription analysis was also carried out in a strain mutated for the *ptsG* gene, which codes for the membrane-bound glucose transporter, EIIBC^{Glc}. The activity of BglG was not affected in the *ptsG* mutant and the proportion of transcripts reading through the terminator is comparable to the wild-type (data not shown). Therefore, although the cytosolic protein EIIA^{Glc} is a mediator of glucose effect in the *bgl* operon, the membrane-bound glucose permease does not affect *bgl* expression.

Discussion

Studies on glucose-mediated repression of several operons encoding genes for the metabolism of non-PTS sugars such as lactose, galactose and maltose have highlighted the presence of intricate mechanisms involved in catabolite repression. However, information on the regulation of one PTS carbohydrate by another is surprisingly lacking in the case of Gram-negative bacteria. In this study, we have attempted to address this problem by examining the effect of glucose on the metabolism of β-glucosides such as salicin and arbutin, which are also taken up by a PTS-dependent permease.

The *bgl* operon of *E. coli* serves as an ideal model system because considerable genetic and molecular information regarding its regulation is already available. Furthermore, expression of the operon has been documented as exhibiting a strong glucose effect (Lopilato & Wright 1990). Our studies have shown

that catabolite repression in the *bgl* operon involves both CRP-cAMP and $EIIA^{Glc}$. However, unlike other systems, transcription initiation is unaffected, and the target of repression is the anti-terminator BglG. The results presented here indicate that, in addition to bringing about induction of the operon by β-glucosides, this regulator is also effectively used to mediate glucose repression of the operon. In nutritionally rich conditions, the activity of BglG is decreased, leading to a dramatic reduction in the proportion of transcripts reading through the *bgl* terminator and reduced expression of the downstream genes. This ensures the preferential utilization of glucose over β-glucosides.

The activity of the anti-terminator protein BglG has been shown to be modulated by EII^{Bgl} (BglF) in response to the availability of β-glucosides by altering its phosphorylation status (Amster-Choder *et al.* 1989; Amster-Choder & Wright 1990, 1992; Schnetz & Rak 1990). Our studies using *bglG* mutants that are insensitive to negative regulation by BglF have shown that, in these under-phosphorylated mutants, glucose is not able to exert its inhibitory effect. This suggests that glucose interferes with the activity of BglG by modulating the BglF-mediated phosphorylation, leading to modification of its function. This is further supported by the observation that glucose effect is markedly reduced in a strain that carries a mutation in *bglF*.

In a recent publication, Gorke & Rak (1999) have presented evidence for the regulation of BglG by antagonistically acting phosphorylations. It has been shown that BglG is phosphorylated by the PTS, independent of the phosphorylation mediated by BglF, which is essential for its activity. Based on a functional analysis of different *pts* mutants, the major PTS component involved in this phosphorylation has been identified as HPr. Thus, phosphorylation mediated by BglF acts negatively and the PTS-mediated phosphorylation acts positively. According to the model proposed by Gorke & Rak, the PTS-mediated phosphorylation of BglG is modulated by the flux of other PTS sugars, which enables utilization of these sugars in a hierarchical manner.

This mechanism of catabolite repression of the *E. coli bgl* operon strongly resembles the regulation of CCR in the *bglPH* operon of *B. subtilis* in which the anti-terminator protein is positively regulated by the PTS proteins, enzyme I and HPr (Kruger *et al.* 1996). In *B. subtilis*, LicT, a member of the BglG/SacY family, brings about anti-termination in the *bglPH* operon and is required for optimal expression of the operon (Kruger *et al.* 1996). Recent studies have shown that the activity of LicT is altered in a nutritionally rich medium by modulation of its phosphorylation status (Lindner *et al.* 1999). Thus, LicT acts as the target for glucose repression in this system, causing premature termination in the presence of glucose. It appears that the regulation of the activity of anti-terminator proteins as a part of the CCR signal transduction process is a common mechanism shared by both Gram-positive and Gram-negative organisms.

The results of our studies using BglF-independent mutants suggest that glucose also influences the BglF-mediated phosphorylation of BglG, since glucose repression is compromised in these mutants. Thus, catabolite repression of the *bgl* operon has multiple components. This is also highlighted by our results which implicate both cAMP-CRP and $EIIA^{Glc}$ in this regulation.

CRP-cAMP is one of the well-studied prokaryotic transcriptional activators, and is involved in regulating a large number of genes, either positively or negatively, in response to carbon nutrient conditions. Bound to its cognate sequence upstream of the target promoters, CRP-cAMP acts to enhance transcriptional initiation by stimulating the steps leading to open complex formation (Malan *et al.* 1984; Ebright 1993; Busby & Ebright 1994; Tagami & Aiba 1995, 1998). An essential characteristic of almost all CRP-dependent promoters is that they are weak promoters and require transcription factors to compensate for the lack of a strong consensus at −10 and −35.

The *bglR* locus has a CRP-cAMP binding site, which bears significant homology to the known CRP binding sites (Reynolds *et al.* 1986). At the same time, the operon harbours a fairly strong promoter which shows significant expression, even in the absence of any activators. The results of our transcriptional analyses show that CRP-cAMP is required for optimal expression of the operon, but this requirement is at the step of transcription termination. This effect of CRP-cAMP on transcription termination is probably not a direct one and does not require binding to its cognate site upstream of the promoter, as transcription initiates normally, even in a *crp cya* mutant. A similar role of CRP in post-initiation regulation was suggested by an earlier study where it was shown to have an anti-polar effect by interfering with rho-dependent terminators in the *lac* and *gal* operons (Ullmann *et al.* 1979).

The enzyme IIA^{Glc}, a cytosolic PTS protein, acts as a sensor for the catabolic state of the cell and in response, inhibits the enzymes for the non-PTS sugar metabolism. The phosphorylation status of this key protein

varies with nutrient conditions and determines the expression of genes like the *lac* operon. Although its role in regulating the uptake and metabolism of non-PTS sugars has been shown unequivocally, whether it affects the metabolism of other PTS sugars was still an open question. Our analysis shows that $EIIA^{Glc}$ acts as the glucose sensor and also mediates repression of the *bgl* operon. However, this effect is distinct from the known role of $EIIA^{Glc}$ in inhibiting non-PTS permeases. A qualitative transport assay using cells which express BglF and BglB constitutively, showed that the uptake of β-glucosides is not decreased due to growth in a glucose-rich medium (data not shown). Thus $EIIA^{Glc}$ does not cause repression of the *bgl* operon by inhibiting the entry of the inducer, salicin, into the cells. Instead, $EIIA^{Glc}$ probably mediates catabolite repression by modulating the activity of BglG.

There is evidence for cross talk between the glucose and β-glucoside transport systems (Schnetz & Rak 1990). $EIIA^{Glc}$ has been shown to phosphorylate site II in EII^{Bgl} (BglF) which further transfers its phoshoryl moiety to BglG. Since our data suggests that BglG is the target for catabolite repression and its activity is altered depending upon the nutritional environment, it is conceivable that $EIIA^{Glc}$ affects *bgl* expression by controlling the phospho-relay: $EIIA^{Glc} \rightarrow EII^{Bgl} \rightarrow$ BglG. However, in glucose grown cultures, $EIIA^{Glc}$ is predominantly found in the unphosphorylated form, while from our analysis, BglG is expected to be phosphorylated as it has lost its anti-termination activity. This rather counterintuitive observation suggests that, although $EIIA^{Glc}$ mediates the glucose effect probably by altering the activity of the anti-terminator, it does so by an indirect pathway and not by controlling the phosphoryl flux described above. One possibility is that the dephosphorylation of BglG by BglF in the presence of β-glucosides is inhibited by $EIIA^{Glc}$ when glucose is being actively transported. This finding needs to be substantiated by additional biochemical experiments.

It is also not clear at this stage whether the regulation mediated by CRP-cAMP is independent of the PTS or whether they converge. One possibility is that CRP-cAMP regulates anti-termination by modulating the levels of the PTS, particularly HPr, and this effect is epistatic over its effect on the expression of $EIIA^{Glc}$.

In conclusion, we have carried out a detailed molecular analysis of the mechanism underlying catabolite repression in the *bgl* operon of *E. coli*. Our analysis indicates that glucose-mediated repression in this system is brought about by modulating the activity of the anti-rerminator, BglG, which in turn controls the expression of the downstream genes. This regulatory system combines features of the mechanisms that are operative in both Gram-positive and Gram-negative bacteria. Interestingly, CRP-cAMP and $EIIA^{Glc}$ are involved in this regulation. The precise molecular details as to how these two different global regulators influence the activity of BglG to bring about glucose effect in the *bgl* operon remain to be elucidated.

Experimental procedures

Plasmids and bacterial strains

The *E. coli* K-12 strains used, along with the relevant description are listed in Table 1A. The plasmids used in this study are listed in Table 1B and are derivatives of pBR322. pA8 was constructed by PCR amplification of the *bglR-G* region. It harbours the insertionally activated regulatory locus and a part of *bglG* up to the *Afl*II site. pANS 5-16 carries the same region, along with the deletion of a G at position +100.

Analysis of transcripts

In vivo transcription analysis was carried out using an S1 nuclease protection assay as described in Singh *et al.* (1995) and Mukerji & Mahadevan (1997). Transformants carrying appropriate *bgl* alleles were grown in M9 minimal medium supplemented with 0.4% glucose or 0.4% succinate and 7 mM salicin as inducer. 40 μg of the RNA was hybridized with a large excess of 5′ end-labelled oligonucleotides complementary to different regions of the *bgl* mRNA. A probe complementary to the β-lactamase mRNA was used as an internal control. All the probes used have a stretch of 6–10 noncomplementary nucleotides at their 3′ end. As a result, after hybridization and digestion with S1 nuclease, bands corresponding to protected transcripts are shorter than the probes used. The digested products were electrophoresed on a 12% acrylamide–urea gel and autoradiographed. The autoradiograms, after appropriate exposure for the bands to be in the linear range, were scanned on a densitometric scanner and the ratio of the net intensity corresponding to the *bgl* and *amp* bands were used for quantification of transcription (*bgl*/*amp*). The *bgl*/*amp* ratio was verified by counting the radioactivity corresponding to the bands using a scintillation counter, for samples showing a large difference in the *bgl* and *amp* signals. The level of glucose effect was determined by taking the ratio of the signal obtained in succinate-grown cultures to that obtained in the glucose-grown cultures.

To rule out the possibility that the *amp* signal varies with growth conditions, glucose-to-succinate ratios were determined using a double internal control involving the *amp* and *kan* transcripts (Mukerji & Mahadevan 1997). These studies showed that there was no variation in the *amp* signal with changes in growth conditions that we employed. The variations in the *amp* signal seen in the different experiments are related to the variations in the mRNA concentrations in different samples and also to the changes in the relative specific activities of the probes

used in the different experiments. Because of this, direct comparisons of the *bgl/amp* ratio have been made only within each panel. This limitation has been overcome by considering the succinate-to-glucose ratios in the different experiments. The glucose effect remains invariant within the limits of experimental error for the different mutants analysed, despite differences in the absolute values of the *bgl/amp* ratios in different experiments.

Assay for β-galactosidase activity

Measurements of β-galactosidase activity were carried out as described by Miller (1972). Cells were grown in M9 minimal medium with 0.4% glucose or 0.4% succinate as the carbon source. Salicin, at a final concentration of 7 mM, was used for induction of the operon.

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