

Growth rate regulation of *lac* operon expression in *Escherichia coli* is cyclic AMP dependent

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Abstract In contrast to the ribosomal RNA gene expression increasing with growth rate, transcription of the *lac* operon is downregulated by cell growth rate. In continuous culture, growth rate regulation of *lac* promoter was independent of carbon substrate used and its location on the chromosome. Since the *lac* operon is activated by cyclic adenosine monophosphate (cAMP), which decreases with increasing cell growth rate, expression of *plac-lacZ* reporter fusion was analyzed in *cya* mutant under various growth conditions. The results demonstrated that expression of *plac-lacZ* in *cya* mutant was both lower and growth rate independent. In addition, ppGpp (guanosine tetraphosphate) was not involved in the mechanism of growth rate regulation of the *lac* promoter. Thus, the results of this study indicate that cAMP mediates the growth rate-dependent regulation of *lac* operon expression in *Escherichia coli*.

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Key words: Growth rate; *lac* operon; Cyclic adenosine monophosphate; *Escherichia coli*

1. Introduction

In *Escherichia coli*, the level of transcription for most genes varies widely according to the nutrient growth condition. The cause of these differences may be the presence of many operon-specific activators or repressors, which vary with the composition of the growth medium. When *E. coli* is grown in minimum medium utilizing different carbon molecules as energy sources, the doubling time is longer in carbon-poor medium than in carbon-rich medium. The regulation of more than 100 individual proteins of *E. coli* exhibits nearly linear variation with growth rate [1,2]. Growth rate-dependent regulation of genes refers to the process that coordinates gene expression with the cellular growth rate. At the transcription level, growth rate affects tRNA synthesis [3], and is involved in the negative feedback regulation of rRNA as well as ribosomal protein synthesis where rRNA and ribosomal protein transcription decrease with decreasing growth rate [4,5]. However, growth rate regulation of fumarate reductase (*frdABCD*), isocitrate dehydrogenase (*icd*), succinate dehydrogenase (*sdhCDAB*), and ATPase (*atpIBEFHAGDC*) genes was identified, showing their expression increases as the cell growth rate decreases [6–9].

The role of cyclic adenosine monophosphate (cAMP) is mediated by the glucose effect on the induction of catabolic enzymes in *E. coli*. The regulatory protein, cAMP receptor protein (CRP), binds cAMP and causes the transcriptional activation or repression of many promoters [10]. The intracellular cAMP concentrations or the ability of the cell to accumulate cAMP are different in different nutrient compositions [11]. Early studies showed that changes in the dilution rate do not vary the cAMP level in *E. coli*, but this may be due to the technical difficulties of measuring low levels of cellular cAMP [12,13]. Recently, Notley et al. have shown that in glucose-limited chemostats, the cells contain elevated cAMP concentrations at low cell growth rates [14,15]. Since the cellular cAMP is a central regulator of hundreds of genes in response to different nutrient states, understanding the cAMP level at different carbon substrates and growth rates is important in understanding the many genes regulated by cAMP in *E. coli*.

The *lac* operon has been considered as the model for understanding the molecular biology of gene expression and its regulation. Transcription of the *lac* operon is regulated by the *lac* repressor and the CRP [16]. Early studies showed that glucose could repress the transcription of the *lac* operon by lowering the intracellular concentration of cAMP [17]. In the absence of glucose, cAMP accumulates inside the cell where it binds to CRP to form the cAMP–CRP complex, which then binds to the CRP binding region located near the *lac* promoter and enhances its transcription. The catabolite repression model is further supported by the observation that glucose can also lower the expression of *crp* gene and reduce the intracellular CRP levels [18]. Physiological regulation of the *lac* operon has been studied by the decontrolled *lac* promoters. The results suggest a small change in β -galactosidase activity with changes in the carbon substrate and growth rate. Most catabolite-insensitive *lac* promoters exhibit a decreased expression only at rapid growth rates when rich media are used [19]. To determine the mechanism of growth rate regulation of the wild-type *lac* promoter, we employed continuous culture to control cell growth rates without varying the types of substrate used. The effect of cell growth rate on expression of *plac-lacZ* reporter fusion under various carbon substrates was then investigated. A strategy using the *cya* gene mutant to explore the role of cAMP on cell growth rate regulation of the *lac* operon in continuous culture was also described. The results of this study showed that the expression of *plac-lacZ* was regulated by the cell growth rate, and this growth rate control of the *lac* operon was dependent on the presence of cAMP in *E. coli*.

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2. Materials and methods

2.1. Bacterial strains, bacteriophages, and plasmids

The genotypes of the *E. coli* strains, bacteriophages, and plasmids are listed in Table 1. *E. coli* strains WM272 and WM265 were kindly provided by Dr. R.W. Simons [20]. Because WM272 and WM265 strains are kanamycin resistant and cannot make the *cya* mutant by P1 transduction, we constructed a new *lac* operon fusion. To construct a wild-type *plac-lacZ* reporter fusion in MC4100, a *lac* operon deletion strain, the 0.36-kb regulatory region of the *lac* promoter was amplified by polymerase chain reaction (PCR) using chromosome DNA of the wild-type *E. coli* K-12 strain W3110. The fragment was cloned into the *EcoRI*–*Bam*HI sites of plasmid pRS415 to give the *plac-lacZ* operon fusion designated pCPT100. The intended fusion junction of pCPT100 was confirmed by DNA sequence analysis. The resulting fusion, containing the 264-bp upstream region of the translational site of the *lacZ* gene and the first 96 bp of *lacZ* DNA, was transferred onto λ RS45 to generate λ CPT100, which was then introduced into MC4100 as previously described by Simons et al. [21]. The *cya*, *crp*, *relA*, and *spoT* deletion strains were constructed by introducing the indicated mutation into strains MC4100 (λ CPT100) containing *plac-lacZ* fusion by P1 transduction followed by selection for resistance to the appropriate drugs [22].

2.2. Cell growth

In batch culture, cells were grown in Luria–Bertani (LB) or in glucose (40 mM) minimal medium (pH 7.0) for β -galactosidase activity assay, unless otherwise indicated. For assay of cells grown on other carbon sources, each compound was substituted at 40 mM. When required, kanamycin and tetracycline were added to the medium at a concentration of 50 mg/l. Aerobic growth was performed as previously described [23]. Flasks or tubes containing the indicated medium were inoculated from overnight cultures grown under the same conditions, and the cells were allowed to double four or five times under exponential phase prior to harvesting for analysis (OD_{600} = 0.40–0.45, Perkin Elmer Lambda Bio20 UV-Vis Spectrometer).

For continuous culture experiments, a New Brunswick BiofloIII fermenter (New Brunswick Scientific Co., Inc.) was fitted with a 1.5-l vessel and operated at a 1-l liquid working volume as previously described [6]. The minimal medium containing 2.25 mM glucose (or acetate) was used to limit cell growth (i.e. carbon-limited medium). Aerobic continuous culture conditions were maintained by saturating the culture medium with sterile air at a rate of 2.0 l/min. To vary the cell growth rate, the medium addition rate was adjusted accordingly. The medium addition rates ranged from 1.67 to 20 ml/min (k = 0.10–1.2/h). When the chemostat was shifted to a new aeration level, steady state was generally achieved within six reactor residence times. This was confirmed by assaying the β -galactosidase activity of harvested

cells as an indicator that gene expression had reached equilibrium. The chemostat was maintained under the same condition until the β -galactosidase values varied no more than 10%. During the experiments, the number of cell doublings per hour (μ) is equal to 1 divided by cell generation time (g). The cell generation (g) is equal to $\ln 2$ divided by cell growth rate (k). Casamino acids were from Difco Co., Detroit, MI, USA. All other chemicals used were of reagent grade.

2.3. β -Galactosidase and cyclic AMP assay

β -Galactosidase levels were determined by hydrolysis of ortho-nitrophenyl- β -D-galactopyranoside (ONPG) as previously described [22,23]. β -Galactosidase values represent the average of at least four independent experiments with a variation of no more than 10% from the mean. ONPG was purchased from Sigma Chemical Co., St. Louis, MO, USA. The samples of *E. coli* MC4100 cells were taken and prepared for assay of the intracellular cAMP levels as described (Amersham Pharmacia Biotech, UK) [15]. The data of the intracellular cAMP concentrations obtained from each continuous culture condition were independently determined at least three times, and cAMP assay variation was less than 10%.

3. Results

3.1. Effect of carbon substrate and growth rate on *lac* operon, *lacUV5* promoter and *plac-lacZ* reporter fusion expression in batch and continuous cultures

Transcription of the *lac* operon is regulated by the *lacI* and the cAMP–CRP complex [16]. While expression of *lacUV5* promoter was shown to be growth rate independent in continuous culture, the *lac* operon expression in wild-type strain W3110 showed three-fold decrease with growth rate (k = 0.10/h vs. k = 1.2/h) (Fig. 1A). Interestingly, when 1 mM isopropyl- β -thiogalactoside (IPTG) was added into the medium to reduce *lacI* repression on *lac* promoter, β -galactosidase activity increased more than 100-fold and showed four-fold downregulation by cell growth rate in wild-type strain W3110, but the expression of *lacUV5* promoter was still not regulated by cell growth rate (Fig. 1B). To determine how the carbon substrate affects *lac* operon expression in the absence of *lacI* gene product, we constructed a wild-type *plac-lacZ* reporter fusion in the MC4100 strain, which is a *lac* operon deletion mutant, and then the cells were grown in LB as well as minimal medium containing various carbon compounds as substrates (glucose, galactose, xylose, succinate, glycerol, and acetate).

Table 1
Bacterial strains, phages and plasmids

Strain/plasmid	Derived from	Genotype/phenotype	Source/reference
Strain			
K12	W3110	wild-type K12 strain	[28]
UV5		HfrH <i>lacL37UV5 thi</i>	[36]
MC4100		F [−] <i>araD139 (argF-lac) U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR</i>	[37]
WM265		DR459 <i>ins93-plac-lacZ</i> ⁺	[20]
WM272		DR459 <i>ins100-plac-lacZ</i> ⁺	[20]
HT28	W3110	Δ <i>cya</i> ::Kan	[38]
IT1409	W3110	Δ <i>crp</i> ::Tet	[38]
CF1693	MG1655	Δ <i>relA</i> ::Kan Δ <i>spoT</i> ::Cml	[39]
CPT100	MC4100	Φ (<i>lac-lacZ</i>) <i>lacY</i> ⁺ <i>lacA</i> ⁺	this work
CPT101	MC4100	Φ (<i>lac-lacZ</i>) <i>lacY</i> ⁺ <i>lacA</i> ⁺ Δ <i>cya</i>	this work
CPT102	MC4100	Φ (<i>lac-lacZ</i>) <i>lacY</i> ⁺ <i>lacA</i> ⁺ Δ <i>crp</i>	this work
Phage			
λ RS45			[29]
λ CPT100	pCPT100	Φ (<i>lac-lacZ</i>) <i>lacY</i> ⁺ <i>lacA</i> ⁺	this work
Plasmid			
pRS415		<i>lacZlacY</i> ⁺ <i>lacA</i> ⁺	[21]
pCPT100	pRS415	Φ (<i>lac-lacZ</i>) <i>lacY</i> ⁺ <i>lacA</i> ⁺	this work
pDIA100	pBR322	expression vector containing adenylate cyclase	[40]

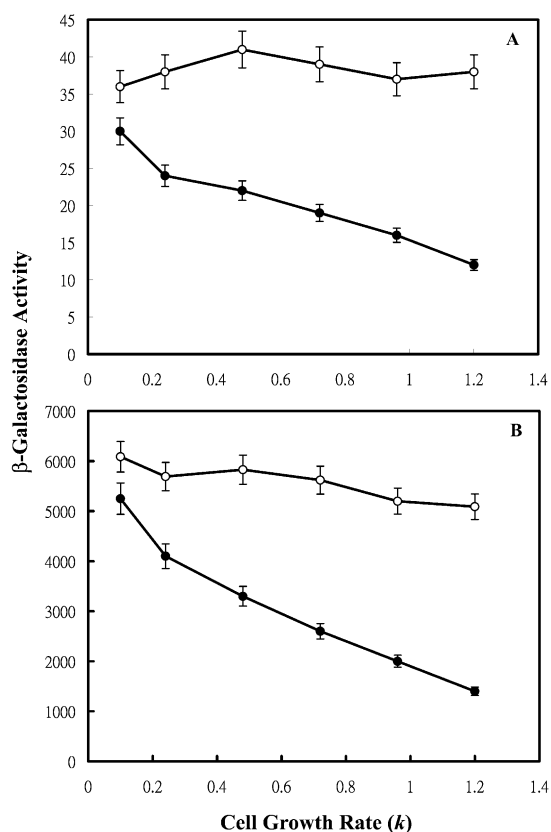


Fig. 1. Effect of cell growth rate on *lac* operon expression of wild-type W3110 strain and the *lacUV5* mutant in continuous culture. A: The cells of wild-type W3110 strain (●) and *lacUV5* mutant (○) were examined in glucose (2.25 mM) minimal medium at the indicated growth rates without IPTG. B: The cells of wild-type W3110 strain (●) and *lacUV5* mutant (○) were grown in glucose (2.25 mM) minimal medium containing 1 mM IPTG at the indicated growth rates. Units are given in nmol of ONPG hydrolyzed per min per mg protein.

Under aerobic condition, expression of *plac-lacZ* varied in the presence of different carbon substrates, whereas it remained low in the *cya* mutant (Fig. 2A). In the next experiment, the cell growth rates in each type of medium were determined, and an inverse relationship between the generation times and *lac* operon expressions was observed using β -galactosidase activities (Fig. 2B). Thus, expression of *lac* operon in response to growth rate was demonstrated. To determine whether the variation of β -galactosidase activity was caused by the types of carbon compound used or the differences in cell growth rate, this inverse relationship was further examined when growth rate was alternatively controlled by a continuous culture using glucose and acetate as carbon substrates. In continuous culture, cell growth rate was controlled by the specific medium flow rate when carbon was limited (i.e. by glucose or acetate). For cultures grown under aerobic condition, the maximum expression of *plac-lacZ* varied more than three-fold, and was higher at a low ($k = 0.10/h$) than at a high ($k = 1.2/h$) cell growth rate. In addition, expression of *plac-lacZ* still exhibited growth rate control when cells were grown in acetate medium (Fig. 3A). This finding demonstrated that growth rate regulation of *lac* operon expression was independent of the carbon substrates used. To assess the *lac* promoter expression at different locations on chromosome could pro-

duce a similar effect, the strains WM265 and WM272 obtained from Dr. R.W. Simons, with the *plac-lacZ* located at 32 and 82 min positions on chromosome, respectively, were also studied in continuous culture [20]. The results of *plac-lacZ* expression were still shown to be growth rate regulated (Fig. 3B). Therefore, growth rate regulation of *lac* operon expression was also independent of its position on the chromosome.

3.2. Effect of the *cya* and *crp* mutations on *plac-lacZ* gene expression in continuous culture

The *lac* operon is controlled by glucose via catabolite repression suggesting that an increase in cell growth rate may increase the glucose uptake. Therefore, the cellular cAMP level would be decreased in theory [16,24]. In this study, the intracellular cAMP levels of the *E. coli* MC4100 strain decreased about three-fold when growth rate increased from $k = 0.10/h$ to $k = 1.2/h$ in glucose minimum medium (Fig. 4). This result was consistent with a three-fold decrease of *plac-lacZ* gene expression in continuous culture. In order to determine the effect of cAMP on growth rate regulation of *lac*

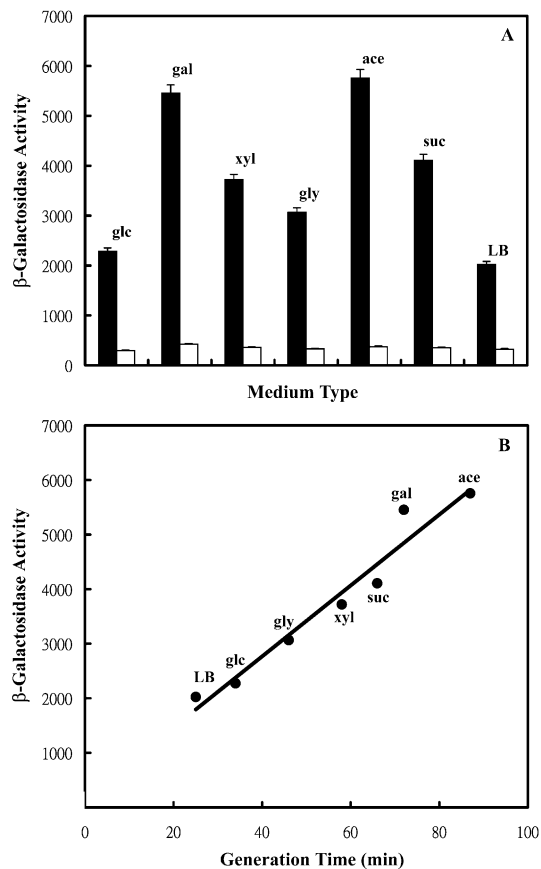


Fig. 2. Effect of carbon substrate, *cya* mutation and cell growth rate on expression of *plac-lacZ* in batch culture. A: MC4100 (λ CPT100) and *cya* deletion mutant cells were grown in minimal medium with the represented carbon compound (40 mM) or in LB medium. The solid bars represent the MC4100 (λ CPT100) and the open bars indicate the *cya* deletion strain. B: MC4100 (λ CPT100) cells were grown in the indicated medium, and the cell generation time as well as β -galactosidase activities were recorded. Medium and carbon compound (40 mM) used for cell growth: glc, glucose; gal, galactose; xyl, xylose; gly, glycerol; ace, acetate; suc, succinate; LB. Units are given in nmol of ONPG hydrolyzed per min per mg protein.

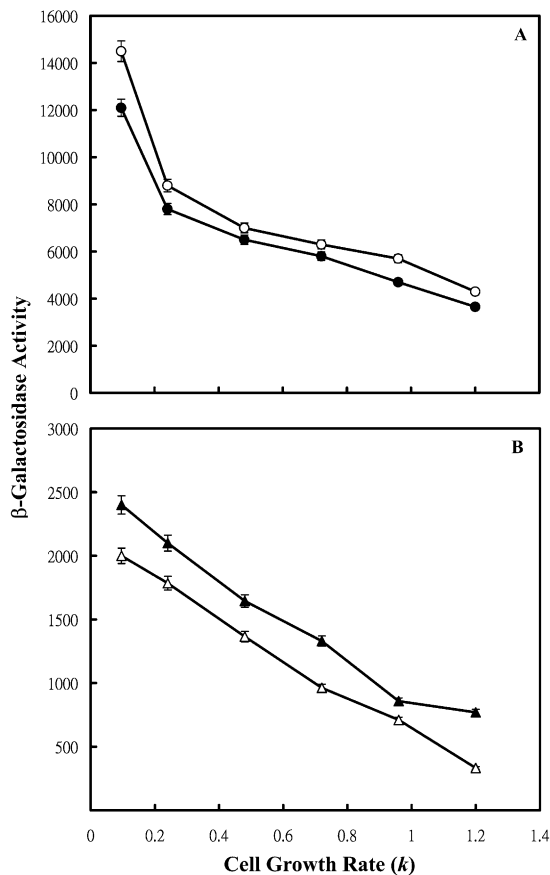


Fig. 3. Effect of cell growth rate on *plac-lacZ* expression in continuous culture. A: MC4100 (λ CPT100) cells were grown with the indicated carbon compounds (2.25 mM) at the indicated growth rates. The carbon compound used for cell growth was glucose (●) or acetate (○) in minimal medium. B: The *plac-lacZ* expression at different locations on chromosome in *E. coli* WM265 (Δ) and WM272 (\blacktriangle) strains, which the *plac-lacZ* located at 32 and 82 min positions of chromosome, respectively, was examined in glucose (2.25 mM) minimal medium at the indicated growth rates. Units are given in nmol of ONPG hydrolyzed per min per mg protein.

operon expression, we created the *cya* and *crp* deletions in MC4100 (λ CPT100) (*plac-lacZ* fusion), using P1 transduction followed by a selection for resistance to the appropriate drug [12]. Thus, the cAMP and CRP could not form a complex in the event cAMP-regulated *lac* operon. As *plac-lacZ* expression in MC4100 was shown to be correlated with the growth rate, the expression was totally abolished in the *cya* and *crp* deletion mutants with glucose and acetate as carbon substrates (Fig. 5A and B). On the other hand, expression of *plac-lacZ* was restored when the plasmid containing *cya*⁺ gene was transformed into the *cya* deletion mutant (Fig. 5A). A similar result was observed in acetate medium (data not shown). These data suggest that growth rate control of *lacZ* gene expression was mediated by the cAMP–CRP complex.

3.3. Effect of ppGpp (guanosine tetraphosphate) on *plac-lacZ* expression in continuous culture

The nucleotide (p)ppGpp, which decreases with cell growth rate, is a positive effector of *lac* operon expression [25]. The *relA* and *spoT* genes, which synthesize ppGpp, have been shown in the regulation of the *lac* operon under amino acid limitation condition [26]. To determine whether ppGpp is also

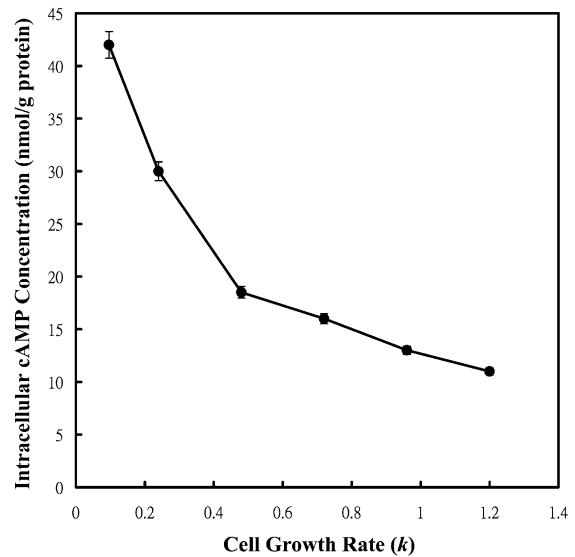


Fig. 4. Effect of cell growth rate on the intracellular cAMP level in continuous culture. *E. coli* MC4100 cells were grown with glucose (●) (2.25 mM) minimal medium at the indicated growth rates. Units are given in nmol per g protein.

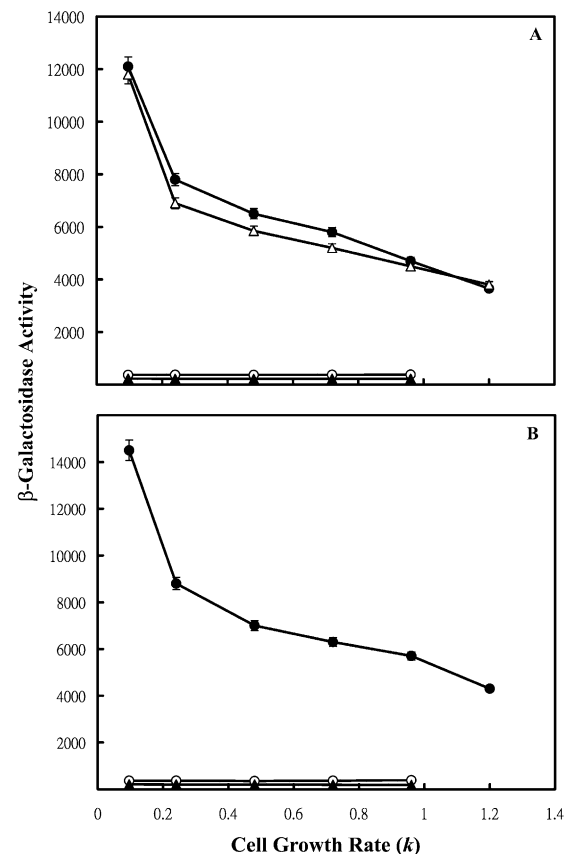


Fig. 5. Effect of cell growth rate and *cya* and *crp* mutations on *plac-lacZ* expression in continuous culture. The cells containing λ CPT100 were grown at the indicated growth rates. A: Strains of MC4100 (λ CPT100) (●), *cya* deletion mutant (○), *crp* deletion mutant (\blacktriangle), and *cya* deletion mutant containing *cya*⁺ plasmid pDIA100 (Δ) were grown in glucose (2.25 mM) minimal medium. B: Strains of MC4100 (λ CPT100) (●), *cya* deletion mutant (○) and *crp* deletion mutant (\blacktriangle) were grown in acetate (2.25 mM) minimal medium. Units are given in nmol of ONPG hydrolyzed per min per mg protein.

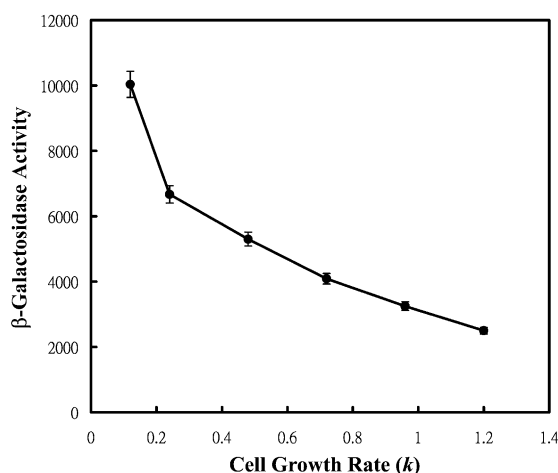


Fig. 6. Effect of *relA spoT* double deletion on *plac-lacZ* expression at different growth rate in continuous culture. MC4100 (λ CPT100) cells containing *relA spoT* double deletion were grown in glucose (2.25 mM) minimal medium (●). Units are given in nmol of ONPG hydrolyzed per min per mg protein.

involved in growth rate regulation of *lac* operon, we created a *relA spoT* double deletion mutant that could not form ppGpp in MC4100 (λ CPT100). When the cells were grown in glucose medium, expression of *plac-lacZ* in *relA spoT* double mutant was about 20% lower than its expression in wild-type MC4100 strain; however, it still exhibited a three-fold growth rate control in continuous culture (Fig. 6). This result indicates that ppGpp is not involved in growth rate regulation of the *lac* operon (Fig. 6).

4. Discussion

Growth rate-dependent regulation of gene expression in *E. coli* falls into two major categories: levels of some genes increase with increasing growth rate, while others decrease [27]. It has been shown that the synthesis rate of ribosomal proteins in *E. coli* is correlated with rRNA transcription and cell growth rate. Although the promoters of rRNA and ribosomal protein genes are stringently controlled by ppGpp, growth rate-dependent control is mechanistically distinct from stringent control in *E. coli* [5,25]. Voulgaris et al. suggest that the relationship between the growth rate control rRNA and feedback regulation processes may be more complicated than currently believed [28]. Expressions of the *gnd* and *dam* genes also increase with growth rate, but the mechanism of regulation is distinct from that of the rRNA and tRNA genes [29,30]. The genes containing gearbox promoter decrease expressions at high growth rates [31], but in this study we have shown that *lac* operon with no gearbox promoter was also downregulated at high growth rates (Figs. 1A, B, 3A and B).

The *lac* operon is subject to positive control by cAMP and CRP as cAMP–CRP binds to the activation site to stimulate *lac* operon transcription [16]. Previous batch culture study on *lac* operon suggests that carbon substrate and growth rate have a small effect on catabolite-insensitive *lacUV5* promoter [19]. In this study, we demonstrated that the *lacUV5* promoter was growth rate independent in continuous culture (Fig. 1A and B), whereas the wild-type *lac* promoter was regulated by cell growth rate and this growth rate control was independent of the carbon substrate and its chromosomal location (Figs.

2A, B, 3A and B). The difference between wild-type *lac* promoter and *lacUV5* promoter is on the -10 region of the RNA polymerase binding site [32]. When the RNA polymerase binds to *lacUV5* promoter and changes structure of the open complex, it may affect the *lacUV5* promoter transcription and reduce the effect of growth rate regulation. Growth rate regulation of the *lac* operon was consistent with a down-regulation of the intracellular cAMP level at high growth rates in strain MC4100 (Fig. 4). Further characterization of *plac-lacZ* expression in the *cya* deletion MC4100 (λ CPT100) strain showed that it was totally abolished but the regulation pattern could be restored when the *cya*⁺ gene was transformed in the cell (Fig. 5A). Therefore, our results suggest that growth rate regulation of *plac-lacZ* expression is cAMP dependent.

The *relA* and *spoT* genes synthesize ppGpp, which is growth rate dependent and involved in regulation of the *lac* operon [26]. However, the results of three-fold growth rate regulation of *plac-lacZ* expression in *relA spoT* double mutant were still seen in this study (Fig. 6). It indicates that ppGpp is not involved in growth rate control of *lac* operon expression. A decrease in FumA and FumC synthesis and catabolite control of the *fumA* and *fumC* promoters were observed when cells were grown in the presence of glucose [33,34]. A recent study has proved that growth rate control of FumA and FumC activities is cAMP dependent [35]. Thus, many promoters, which have their expressions affected by cAMP, may be growth rate dependent in *E. coli*.

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