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.

Keywords: 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 energy sources, the doubling time is longer in carbon-poor medium utilizing different carbon molecules as energy sources. When E. coli is grown in carbon-poor medium, the level of transcription for most genes is reduced 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 lac operon was 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]. 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 chromosomal DNA of the wild-type E. coli K-12 strain W3110. The fragment was cloned into the EcoRI-BamHI 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 λRS45 to generate λCPT100, 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 (λCPT100) 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 μg/ml. 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 (OD600 = 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 1.5-1 vessel and operated at a 1-1 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 (g) is equal to 1 divided by cell generation time (t). The cell generation (t) 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 Pharmaea 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>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Derived from</th>
<th>Genotype/phenotype</th>
<th>Source/reference</th>
</tr>
</thead>
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<tr>
<td>K12</td>
<td>W3110</td>
<td>wild-type K12 strain</td>
<td>[28]</td>
</tr>
<tr>
<td>UV5</td>
<td></td>
<td>HfrH lacL7UV5 thi</td>
<td>[36]</td>
</tr>
<tr>
<td>MC4100</td>
<td></td>
<td>F’ araD139 (argF-lac) U169 rpsL150 relA1 fIbB5301 deoC1 ptsF25 rbsR</td>
<td>[37]</td>
</tr>
<tr>
<td>WM265</td>
<td></td>
<td>DR459 mis93-plac-lacZ</td>
<td>[20]</td>
</tr>
<tr>
<td>WM272</td>
<td></td>
<td>DR459 mis100-plac-lacZ</td>
<td>[20]</td>
</tr>
<tr>
<td>HT28</td>
<td>W3110</td>
<td>Δcya::Kan</td>
<td>[38]</td>
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<tr>
<td>IT1409</td>
<td>W3110</td>
<td>Δcrp::Tet</td>
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<td>MG1655</td>
<td>ΔrelA::KanΔspoT::Cml</td>
<td>[39]</td>
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<td>MC4100</td>
<td>Φ(lac-1acZ) lacYlacA</td>
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<tr>
<td>CPT102</td>
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<tr>
<td>pDIA100</td>
<td>pBR322</td>
<td>expression vector containing adenylate cyclase</td>
<td>this work</td>
</tr>
</tbody>
</table>

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 L-galactosidase activities (Fig. 2B). Thus, expression of lac operon in response to growth rate was demonstrated. To determine whether the variation of L-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 produce 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...
operon expression, we created the cya and crp deletions in MC4100 (λCPT100) (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 (pppGpp, 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. 3. Effect of cell growth rate on plac-lacZ expression in continuous culture. A: MC4100 (λCPT100) 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 (△) and WM272 (▲) 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.

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 λCPT100 were grown at the indicated growth rates. A: Strains of MC4100 (λCPT100) (●), cya deletion mutant (□), crp deletion mutant (▲), and cya deletion mutant containing cyar plasmid pDIA100 (△) were grown in glucose (2.25 mM) minimal medium. B: Strains of MC4100 (λCPT100) (●), cya deletion mutant (□) and crp deletion mutant (▲) were grown in acetate (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 (ΔCPT100). 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 bound to 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 downregulation 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 (ΔCPT100) 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 was 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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References


Fig. 6. Effect of relA spoT double deletion on plac-lacZ expression at different growth rate in continuous culture. MC4100 (ΔCPT100) 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.