Aspects of the Regulation of Adenylate Cyclase Synthesis in *Escherichia coli* K12

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In *Escherichia coli* K12 expression of the adenylate cyclase gene is subject to multiple controls. In order to gain understanding of the regulation of adenylate cyclase synthesis, operon and protein fusions were constructed by *in vitro* recombination either into bacteriophage λ or low-copy-number plasmids, or directly on the chromosome at the *cya* locus. The fusions were used in physiological experiments as probes to study transcriptional and translational controls of *cya* expression. It was found that adenylate cyclase synthesis was insensitive to glucose effects. As already described by other workers, the CAP–cAMP complex had a moderate negative control on *cya* expression. In addition it was observed that concomitant with a severe slackening of growth rate, specific to the growth of *cya* strains in rich medium, *cya* expression was considerably enhanced. This increase of adenylate cyclase synthesis did not appear to be directly dependent on the presence of a functional cAMP receptor (CAP), and seemed to be controlled at the level of transcription. Finally, translation of the *cya* message was very weak when compared to *cya* transcription (the mRNA level was the same in protein and operon fusions).

INTRODUCTION

In bacteria, adenosine 3':5'-cyclic monophosphate (cAMP) controls the synthesis of many enzymes and various membrane-associated proteins (Botsford, 1981; Ullmann & Danchin, 1983). Such control depends on the nature of the carbon source and requires the presence of a functional cAMP receptor protein, CAP (Botsford, 1975; Epstein *et al.*, 1975; Wayne & Rosen, 1974). In *Escherichia coli*, cAMP is also involved as a modulator of catabolite repression: low cAMP levels are correlated with a severe reduction in the synthesis of enzymes involved in carbon catabolism. In addition, the cAMP–CAP complex is involved in transcription termination of several polycistronic operons (Ullmann *et al.*, 1979; Guidi-Rontani *et al.*, 1984). However, whereas the molecular mode of action of the cAMP–CAP complex seems relatively well understood (De Crombrughe *et al.*, 1984), the processes controlling variations of intracellular cAMP levels remain unclear. In fact, it has been shown that cAMP is very efficiently excreted from the cell (Matin & Matin, 1982), and that phosphodiesterase is poorly active in *E. coli* K12. It has also been shown that *crp* strains overproduce cAMP (Potter *et al.*, 1974; Wayne & Rosen, 1974). This led Botsford & Drexler (1978) to postulate that CAP in its active conformation (i.e. bound to cAMP) might act as a repressor of the adenylate cyclase gene, *cya*. Experimental evidence has recently been accumulated which substantiates the latter hypothesis (Aiba, 1985; Kawamukai *et al.*, 1985). However, the actual repression of *cya*

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Abbreviations: CAP, cAMP receptor protein; ONPG, *o*-nitrophenyl β -D-galactoside; X-gal, 5-bromo-4-chloro-3-indolyl β -D-galactoside.

transcription by cAMP has been found to be weak (two- to sixfold); thus it would hardly explain the large effects observed *in vivo* (30- to 100-fold increase in *crp* strains) (Joseph *et al.*, 1982). To elucidate the control of cAMP synthesis, it appeared necessary to investigate the details of *cya* expression. The *cya* genes of *E. coli* (Roy & Danchin, 1982; Aiba *et al.*, 1983, 1984) and of *Salmonella typhimurium* (Wang *et al.*, 1981) have been cloned and characterized, and *cya* gene expression has been studied. In particular, *cya-lacZ* fusions on multicopy plasmids were constructed, and preliminary physiological results questioned the usual interpretations of the glucose and cAMP-CAP controls of *cya* expression (Roy *et al.*, 1983*b*). In the present paper we document a physiological analysis of the expression of *cya-lacZ* operon and protein fusions in different *E. coli* backgrounds, when present as single copies relative to the chromosome copy number. The fusions were introduced either as bacteriophage λ prophage, as low-copy-number plasmids, or directly within the chromosome, and then used as probes to study the transcriptional and translational regulation of *cya* expression.

METHODS

Bacterial and phage strains, and growth media. The bacterial and bacteriophage strains used are listed in Table 1. The usual growth media were the rich medium LB (Miller, 1972), the minimal medium M68 (Ullmann *et al.*, 1976) or the synthetic medium MM1: NaCl 100 mM, $(\text{NH}_4)_2\text{SO}_4$ 10 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 5 mM, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1 mM, KH_2PO_4 8 mM, K_2HPO_4 16 mM, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 10 μM , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1 μM , $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.1 μM , $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.1 μM , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.1 μM , $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ 0.1 μM , CrCl_3 0.1 μM , NiCl_2 0.1 μM , supplemented with 0.4% carbon source (glucose or glycerol), 5 μg thiamin ml^{-1} and 0.1% Casamino acids. When included, 5-bromo-4-chloro-3-indolyl β -D-galactoside (X-gal) was added at 40 μg ml^{-1} , and cAMP at 3 mM. R medium was as in Miller (1972); RMM is R medium with addition of 5 mM- MgSO_4 and 0.2% maltose.

Construction of λ recombinants carrying *cya-lacZ* fusions. λ recombinants were constructed *in vitro*. In the case of operon fusions the respective *EcoRI* DNA fragments of the different *cya-lac* fusions (Fig. 1) carried by pBR322 derivatives were introduced into bacteriophage λ gt4 DNA digested with *EcoRI*. In the case of the protein fusions, the *EcoRI*-*SacI* fragment of each protein fusion plasmid was ligated with the right arm of λ gt4 digested with *EcoRI* and the left arm of λ sew digested with *SacI*. λ recombinant DNA was subsequently packaged *in vitro* (Hohn, 1979). Lysogeny with phages carrying the *cya-lacZ* fusions was performed by infecting bacteria at an m.o.i. of 0.1 (in order to obtain preferentially monolysogens), preadsorbing for 10 min at 30 °C and plating directly on RMM/X-gal plates containing cAMP in the presence of the λ clear phages W30 (λ) and W248 (λ h80). In the case of *crp* strains, which lack λ receptor, lysogeny was performed by phenotypic mixing of phages carrying the fusions, with λ W248 (h80) (the h80 host receptor synthesis is insensitive to the absence of an active cAMP-CAP complex). For each experiment five to ten lysogens were purified and tested for β -galactosidase activity. The lowest value was taken as that of a monolysogen.

Construction of low-copy-number plasmids carrying *cya-lacZ* fusions. For rapid screening of recombinants, small-scale plasmid (Birnboim & Doly, 1979) and phage (Davis *et al.*, 1980) DNA preparations were employed. Large quantities of plasmid DNA were obtained as previously described (Roy & Danchin, 1982). In order to introduce

Table 1. Bacterial and bacteriophage strains

Strain	Genotype	Source
Bacteria		
TP2100	F ⁻ <i>xyl lacΔX74</i>	Roy & Danchin (1982)
TP2006	F ⁻ <i>xyl cyaΔ lacΔX74 glp*</i>	Roy & Danchin (1982)
TP2339	F ⁻ <i>xyl cyaΔ crpΔ39 lacΔX74 argH1</i>	Roy <i>et al.</i> (1983 <i>b</i>)
TP 2065	F ⁻ <i>xylΦ(cya-lac) lacΔX74 argH1</i>	This study
XA102	F ⁻ (<i>lac-pro</i>)Δ <i>nalA rpoB metB argEam supE ara</i>	Coulondre & Miller (1977)
XA103	F ⁻ (<i>lac-pro</i>)Δ <i>nalA rpoB metB argEam supF ara</i>	Coulondre & Miller (1977)
Bacteriophages		
λ gt4	<i>cI857 nin5</i>	Davis <i>et al.</i> (1980)
λ sew	<i>cI857 nin5 Wam403 Eam1100 Sam100</i>	Enquist <i>et al.</i> (1976)
λ 1872 or 1873 or 1873	<i>cI857 nin5 Φ(cya-lac⁺)</i>	This work, Figure 1
λ 1865	<i>cI857 nin5 Wam403 Eam1100 Φ(cya-lac⁺)</i>	This work, Figure 1
λ 1861	<i>cI857 nin5 Wam403 Eam1100 Φ(cya⁺-lac⁺)</i>	This work, Figure 1

*glp** is a mutation located very near the *argH* gene; it allows growth of a *cya* strain with glycerol as carbon source.

the *cya-lacZ* fusions into the low-copy-number plasmid pDIA13 (a broad-host-range plasmid belonging to the IncW incompatibility group: De Reuse *et al.*, 1986), the *EcoRI*–*SalI* DNA fragment of each multicopy plasmid (Fig. 1) was digested and ligated with pDIA13 digested with the same restriction enzymes. Transformation was performed as previously described (Roy & Danchin, 1982) and transformants were selected on MacConkey agar plates containing 1% (w/v) lactose and kanamycin (25 µg ml⁻¹) as required.

Introduction of a *cya-lacZ* fusion into the *E. coli* chromosome. The short protein fusion (86 amino acids of adenylate cyclase) was introduced into the chromosome in place of the *cya* gene as follows. Plasmid pDIA1780 (see Fig. 2), containing a 7 kb fragment carrying the *cya-lacZ* protein fusion followed by the end of the putative *cyaX* gene, was used as a starting material. After restriction with *EcoRI*, the fragment devoid of a replication origin, carrying an intact tetracycline-resistance gene and the *cya-lacZ* fusion, was purified and ligated. It was then used to transform strain TP2100, and tetracycline-resistant clones, having integrated the circularized fragment by homologous recombination, were selected on LB plates containing X-gal (40 µg ml⁻¹) and tetracycline (7 µg ml⁻¹). The next step was selection for Cya⁻ recombinants using a virulent λ phage as selective agent. Such clones were obtained at a frequency of 10⁻³, consistent with the expected frequency for homologous recombination (spontaneous resistance to λ *vir* occurs at a frequency lower than 10⁻⁶). As expected, all recombinants had lost the tetracycline-resistance gene and were sensitive to the antibiotic. The resulting strain carried a *cya-lacZ* fusion in place of the wild-type *cya* gene. In addition the 5' end of the putative *cyaX* gene was deleted.

β -Galactosidase assays. β -Galactosidase activity was determined by the method of Pardee *et al.* (1959). One unit (U) of β -galactosidase was defined as the amount of enzyme that hydrolysed 1 nmol ONPG min⁻¹ at 28 °C. Strains characterized phenotypically were grown in the appropriate minimal medium for at least eight generations. Each experimental point shown represents the average of activities determined in three or more independent cultures. Samples from cultures were subsequently streaked on glucose/X-gal plates in order to confirm that spontaneous curing of λ or plasmid *cya-lac* fusions was negligible.

RNA–DNA hybridization. Cells carrying *cya-lacZ* recombinant bacteriophages were grown at 30 °C in medium M68 containing 0.4% glucose and 0.2% Casamino acids, in the absence or presence of 3 mM-cAMP. The cells were pulse-labelled for 2 min with [5,6-³H]uridine [1 mCi ml⁻¹, 46 Ci mmol⁻¹ (1.7 TBq mmol⁻¹)] and RNA was extracted as described by Hagen & Young (1978). The yield was of the order of 5 × 10⁷ c.p.m. per mg RNA and was independent of the presence of cAMP in the growth medium.

Plasmid pDIA9001 carrying the *lacZ* gene (Guidi-Rontani *et al.*, 1984) was digested by *EcoRI* and denatured with alkali. The DNA was bound to filters according to Gillespie & Spiegelman (1965) (nitrocellulose filters, Schleicher and Schuell, no. BA85, 0.45 µm pore size). Microfilters of 6 mm diameter carrying 1–15 µg plasmid DNA were prepared for each experiment. Filter hybridizations were performed as described by Kourilsky *et al.* (1974). RNA hybridization in the presence of excess DNA directly determines the total number of counts in the sample, corresponding to maximum level of RNA which can hybridize to the probe.

Reagents. Restriction enzymes, T4 DNA ligase and cAMP were obtained from Boehringer Mannheim, pancreatic T1 RNAase, lysozyme, tetracycline, kanamycin and ampicillin from Sigma, ONPG and X-gal from Serva, [5,6-³H]uridine from Amersham and phenol from Carlo Erba.

RESULTS AND DISCUSSION

Expression of cya-lac fusions in minimal medium: low translation efficiency of the cya message

Fig. 1 shows the organization of the *cya* gene, which comprises a long open reading frame (2544 bp), following two promoter regions, P1 and P2 (Roy *et al.*, 1983; Aiba *et al.*, 1984; Aiba, 1985). In order to avoid fluctuations in the plasmid copy number, known to occur with ColE1 derivatives and to interfere with cAMP-mediated controls (Botsford, 1981), the DNA segments containing the *cya-lacZ* fusions were introduced either into bacteriophage λ or on plasmids derived from the IncW replicon, as shown in Fig. 1. In addition one of the protein fusions (Fig. 2) was directly introduced into the chromosome in place of the *cya* gene (strain TP2065).

The effect of extracellular cAMP on β -galactosidase synthesis was investigated in strains containing the different *cya-lacZ* fusions. At concentrations ≥ 3 mM, cAMP had a negative effect, not varying with cAMP concentration, on β -galactosidase synthesis; a smaller negative effect was detected at 1 mM-cAMP. The strains harbouring the different λ recombinants were therefore grown in glucose minimal medium supplemented with 3 mM-cAMP, and under such conditions β -galactosidase synthesis was repressed by a factor of 1.8 for λ 1872, 2.4 for λ 1877 and 3 for λ 1873. A similar repression ratio of 3 was found with the protein fusion, λ 1865. Thus a relatively small negative effect of cAMP was observed on the expression of both operon and protein fusions, more significant when both *cya* promoter regions were present (λ 1873 and

Fig. 1. Restriction map of the *cya* gene and *cya-lacZ* fusions. pDIA1872, pDIA1873, pDIA1877 are operon fusions and pDIA1865 and pDIA1861 are protein fusions (Roy *et al.*, 1983b). The heavy line represents the *lac* operon DNA, the open line DNA from pBR322 and the thin line *cya* DNA. □, *Eco*RI; ●, *Bam*HI; ▲, *Hpa*I; ◇, *Sal*I; △, *Sac*II.

**Eco*RI fragment of the respective multicopy plasmids. †*Eco*RI-*Sac*II fragment of either pDIA1865 or pDIA1861. ‡*Eco*RI-*Sal*I fragment of either operon or protein fusions.

Fig. 2. Restriction map of plasmid pDIA1780. ▽ represents the junction between the *Eco*RI site at position 3471 in *cyaX* and the *Nru*I site at position 4980 in *lacA*. ▢ represents an *Eco*RI site obtained after ligation of the 'filled in' *Eco*RI site located upstream from the *cya* gene with the *Pvu*II site of pBR322. ◇ represents a *Sal*I site obtained after ligation of the filled *Sal*I site downstream from *cya* locus with the *Pvu*II site of pBR322. Other symbols are as in Fig. 1.

1865). As can be seen in Table 2, this effect is mediated by CAP, since β -galactosidase synthesis did not vary in a *crp* strain (TP 2339) whether in the presence or in the absence of cAMP. It can also be noticed that *cya* translation efficiency was very weak when compared to the corresponding transcription level (compare the 1865 construction and the 1873 construction).

The cAMP-mediated negative control, monitored indirectly by measurement of β -galactosidase activity, was also investigated directly by measuring mRNA synthesis. Total cellular mRNA from strains TP2006 λ 1865 and TP2006 λ 1873, labelled with [3 H]uridine, was hybridized to increasing concentrations of *lacZ'* DNA (*Eco*RI restriction fragment from pDIA9001) bound to nitrocellulose filters (Table 3). There was a 1.5-fold excess of the operon fusion mRNA synthesized in the presence of cAMP, as compared to the amount synthesized in the absence of the nucleotide. This is quite similar to the ratio observed by measuring β -galactosidase activities, and in agreement with the results of Aiba (1985) and Kawamukai *et al.* (1985). The synthesis of mRNA did not vary significantly between the operon fusion and the protein fusion (Table 3).

A puzzling observation has to be emphasized here: translation of the *cya* message seems to be extremely inefficient. Indeed the level of protein fusion expression was decreased more than

Table 2. Expression of *cya-lacZ* fusions in the presence and in the absence of cAMP

Strain	Bacteriophage	β -Galactosidase activity (U per OD ₆₀₀ unit)		Repression ratio*
		No cAMP	3 mM-cAMP	
TP2006 (<i>cya</i> , <i>crp</i> ⁺)	λ 1872 (operon fusion, P1)	103	54	1.8
	λ 1877 (operon fusion, P2)	909	367	2.4
	λ 1873 (operon fusion, P1, P2)	954	294	3
	λ 1865 (protein fusion, P1, P2)	4	1.4	3
	λ 1861 (protein fusion, P1, P2)	1.5	ND	
TP2339 (<i>cya</i> , <i>crp</i>)	λ 1873 (operon fusion, P1, P2)	730	730	1
	λ 1865 (protein fusion, P1, P2)	4	4	1

ND, Not determined.

* (Units of β -galactosidase activity of strains grown in MM1 with glucose as carbon source without cAMP)/(units of β -galactosidase activity of strains grown in the same medium with 3 mM-cAMP).

Table 3. Synthesis of *cya-lacZ*-specific mRNA in the absence and in the presence of cAMP

Strain TP2006 (*cya*, *lac*) harbouring the bacteriophage indicated was grown at 30 °C in M68 medium supplemented with 0.4% glucose and 0.2% Casamino acids in the presence or absence of 3 mM-cAMP, and pulse-labelled for 2 min with [³H]uridine as described in Methods. The respective values given in the columns were obtained from the same RNA preparations using the same amount of TCA-precipitable activity. Non-specific hybridization was determined using RNA from strain TP2006, in which the entire *lac* operon is deleted.

Bacteriophage	Hybridization (% of total input)	
None	0.001	0.001
λ 1873 (operon fusion, P1, P2)	0.27	0.17
λ 1865 (protein fusion, P1, P2)	0.24	0.17

100-fold when compared with the level obtained in operon fusions (Table 2; see also Table 5). In contrast, the total amount of mRNA measured from operon or protein fusions was similar (Table 3). Both *cya-lacZ* chimaeric proteins displayed the same level of β -galactosidase activity, suggesting that they had the same turnover number for ONPG. The larger protein has been purified to homogeneity (Danchin *et al.*, 1984), and has been shown to have a turnover number identical to that of wild-type β -galactosidase and to be cytoplasmic. Thus it can be excluded that the low level of activity was due to some conformational artifact (see Ullmann, 1984). In addition, the chimaeric proteins are stable under different growth conditions (data not shown). The poor expression of *cya* therefore seems to be due to a post-transcriptional effect. No special sequence feature (such as palindromes, or a promoter permitting synthesis of anti-mRNA) seems obvious in the case of *cya*, and so we are left with speculations about the nature of the *cya* translational control. The only relevant feature in the sequence is that the open reading frame starts with a UUG codon, which is rather rare in Gram-negative bacteria, and which, at least according to Stormo's rule (Stormo *et al.*, 1982), should be a poor initiator. However, several proteins that are expressed at a fairly high level appear to have a UUG start codon (including a ribosomal protein S20: Mackie, 1981). In addition, replacement of UUG by an AUG codon did not drastically affect *cya* expression (unpublished results; Reddy *et al.*, 1985). Experiments are in progress to clarify this issue.

Effect of carbon source

Cellular levels of cAMP are known to fluctuate as a function of the nature of the carbon source on which the strain is grown (Botsford & Drexler, 1978; Epstein *et al.*, 1975). But Joseph *et al.* (1982) showed that when bacteria were grown on a variety of carbon sources, adenylate cyclase activity was inversely related to the intracellular concentration of cAMP. In addition it has been observed (Guidi-Rontani *et al.*, 1980; Ullmann & Danchin, 1983), that catabolite repression can be separated from the cAMP effect, and is a function of the nature of the carbon source (independently of cAMP synthesis). It was therefore of interest to measure the variations of adenylate cyclase synthesis, as a function of the carbon source, using β -galactosidase as a probe, in the absence of cAMP.

Strain TP2006 harbouring bacteriophage λ carrying either operon or protein fusions was grown in minimal medium supplemented either with glucose or with glycerol as carbon source, and β -galactosidase activity was determined (Table 4). This strain was used because, although defective in cyclase activity, and thus unable to grow on a variety of carbon sources such as succinate, it has a mutation in the glycerol operon, allowing growth on glycerol. The highest β -galactosidase activity was observed when bacteria were exponentially growing on glucose. In the presence of glycerol, slightly lower levels of β -galactosidase activity were observed when cAMP was absent from the medium, suggesting that adenylate cyclase synthesis is not repressed by glucose. Moreover, no effect of cAMP was observed on expression of either protein or operon fusions, when strains were grown in minimal medium containing glycerol as a carbon source.

*Expression of *cya-lac* fusions in rich medium*

Strains derived from the TP2000 background grow very slowly in minimal medium and are (because of their *cya* mutation) unable to utilize carbon sources other than glucose (or glycerol in the case of TP2006). Expression of the fusions was therefore studied with bacteria grown in rich medium (at 30 °C in the case of bacteria harbouring thermosensitive bacteriophage λ , otherwise at 37 °C). A *cya*-specific effect was observed under such conditions: the *cya* strain showed an abrupt decrease in growth rate at a density (OD_{600} 0.9) far from normal saturation (OD_{600} 4–5) when compared with a wild-type counterpart, or when cAMP is added to the growth medium. There was a concomitant increase of β -galactosidase synthesis (Fig. 3): it increased 5- to 10-fold in each *cya-lacZ* fusion in the absence of cAMP. Contrariwise, strains grown in the presence of cAMP presented a longer phase of rapid growth and no increase of β -galactosidase synthesis could be observed (Fig. 3, Table 5). Similar results were obtained when using fusions carried either on λ or on low-copy-number plasmids. These observations were specific to *cya* expression: for instance, no similar increase of β -galactosidase synthesis could be observed with a fusion between *cro* and *lacZ* placed under control of the right λ promoter (pDIA3237; De Reuse *et al.*, 1986). Such an increase of adenylate cyclase synthesis was also observed when the protein fusion was integrated at the *cya* locus on the chromosome (strain TP2065). The increase in β -galactosidase synthesis was always strictly related to the growth state of the strain (shown by dilution experiments). The reason for the abrupt change in growth rate of *cya* strains is unexplained. Several parameters were therefore investigated. No significant pH variations could be observed during growth, and addition of 0.5% Casamino acids did not restore a longer phase of rapid growth. Starvation for carbon source might be suspected as a cause of this growth rate slackening. Different carbon sources such as glucose, glucose 6-phosphate, fructose, glycerol, pyruvate, sodium acetate and 2-oxoglutarate were therefore tested. These different carbon source supplies delayed, to a greater or lesser extent, the entry into a slow growth phase; as a result of this delay the increase in β -galactosidase synthesis was abolished (at least in part). Finally, the cAMP and glucose effects were synergistic, as they restored β -galactosidase synthesis to the level measured during the fast growth phase (Fig. 3).

Thus, in a *cya* strain grown in rich medium a 5- to 10-fold increase in β -galactosidase synthesis, concomitant with a dramatic change in growth rate, was observed. No increase was seen when the rapid growth phase was maintained, either after cAMP addition or after addition of carbon sources such as glucose or glucose 6-phosphate. The effect of cAMP on growth was mediated by CAP, as it was absent in *crp* mutants; it seems unlikely that it resulted from a direct

Fig. 3. Growth curve (a) and rate of β -galactosidase synthesis (b) of strain TP2006 λ 1873 in rich medium. ●, LB; ■, LB + cAMP or LB + glucose; ▲, LB + fructose.

Table 4. Effect of carbon source on expression of *cya-lacZ* fusions in strain TP2006 (*cya*, *lac*)

Bacteriophage	cAMP (3 mM)	β -Galactosidase activity (U per OD ₆₀₀ unit)		Repression ratio*
		Glucose	Glycerol	
λ 1873	—	910	480	1.8
(operon fusion, P1, P2)	+	370	480	0.7
λ 1865	—	4.2	3	1.4
(protein fusion, P1, P2)	+	1.5	1.5	1

* (Units of β -galactosidase activity of strains grown in MM1 with glucose as carbon source)/(units of β -galactosidase activity of strains grown in MM1 with glycerol).

Table 5. Expression of *cya-lacZ* fusions in rich medium

cAMP (3 mM) . . .	β -Galactosidase activity (U per OD ₆₀₀ unit)		
	Exponential growth + or —	Stationary growth	
		—	+
Operon fusions P1, P2*			
λ 1873	450	2000	660
pDIA 1973	640	5000	1000
Protein fusions P1, P2*			
λ 1865	2	27	2
λ 1861	2	2	ND
pDIA1965	2	36	5
pDIA1961	2	4	ND
TP2065†	1	5	1
TP2006(pDIA3237)‡	240	240	240

ND, Not determined.

* Operon and protein fusions were in strain TP2006 (*cya*, *lac*).

† TP2065 is the *cya-lacZ* protein fusion on the chromosome.

‡ Plasmid pDIA3237 is a *cro-lacZ* protein fusion under the control of λ p_R.

action of cAMP, since the same effect was observed with glucose, in the absence of cAMP. The enhancement of adenylate cyclase synthesis observed when cells reduced their growth rate was controlled at the level of transcription (it did not vary between protein and operon fusions). Unfortunately this effect could only be demonstrated when bacteria were grown in a rich medium (LB), and it has not yet been possible to make up a minimal medium where a similar effect can be observed. Indeed in all conditions tested (limitations in carbon or phosphate supply, growth in the absence of oxygen, etc.) cells behaved as if they expressed a partially derepressed *cya* gene. Further work is therefore needed in order to identify the metabolite(s) responsible for this control of *cya* expression.

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