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Transcriptional Regulation of *Escherichia coli serC-aroA* Operon : Further Support for cAMP-Dependent Expression

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Abstract: The *Escherichia coli* mixed-function *serC-aroA* operon encodes biosynthethic enzymes for unrelated pathways leading to the syntheses of serine and aromatic amino acids. It has been proposed that the operon is expressed in a cAMP-dependent manner. In this work experiments were performed to investigate the cAMP-dependent expression of the operon. Exogenous cAMP increased β-galactosidase synthesis in the *cya*$^+$ and *cya*$^-$ strains harboring the *serC-aroA-lac* fusion plasmid. This enhancement was more dramatic in the *cya*$^-$ strain grown in a minimal medium. In a dot blot assay the *serC-aroA* mRNA content increased in a concentration-dependent pattern after the addition of exogenous cAMP. The activity of phosphoserine aminotransferase, encoded by the *serC* gene, apparently increased in *E. coli* cells after the addition of cAMP. All results obtained confirmed that the expression of the *E. coli serC-aroA* operon is positively regulated by cAMP at the level of transcription.

Key words: *Escherichia coli*, *serC-aroA* operon, cAMP, dot blot assay.

The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase; EC 2.5.1.19) catalyzes a reaction in the biosynthetic pathway leading to chorismate. This is the only pathway in bacterial, fungal, and plant species for the biosynthesis of aromatic compounds, including aromatic amino acids. EPSP synthase is the primary target of the nonselective herbicide glyphosate (Boocock & Coggins, 1983; Comai *et al.*, 1983; Stalker *et al.*, 1985).

The *aroA* genes encoding EPSP synthase have been cloned from various organisms including: *Escherichia coli* (Duncan *et al.*, 1984), *Salmonella typhimurium* (Comai *et al.*, 1985), *Salmonella gallinarum* (Griffin & Griffin, 1991), *Mycobacterium tuberculosis* (Garbe *et al.*, 1990), *Bordetella pertussis* (Maskell *et al.*, 1988), *Aspergillus nidulans* (Charles *et al.*, 1986), *Yersinia enterocolitica* (O'Gaora *et al.*, 1989), *Saccharomyces cerevisiae* (Duncan *et al.*, 1987) and higher plants, such as petunia and tomato (Grasser *et al.*, 1988).

In *E. coli* (Duncan & Coggins, 1986) and *S. typhimurium* (Hoiseth & Stocker, 1985) the *aroA* gene is a part of an operon which includes the *serC* gene. The *serC* gene encodes the enzyme phosphoserine aminotransferase (PSAT; EC 2.6.1.52) which is involved in the serine biosynthetic pathway. Serine and chorismate, the end products of the two pathways, are precursors of enterochelin, a siderophore required for the uptake of iron from the environment. Although EPSP synthase and PSAT, which catalyze reactions on separate pathways, are synthesized constitutively (Tribe *et al.*, 1976; McKitrick & Pizer, 1980), the precise regulation of the *serC-aroA* operon is not known.

The *serC* and *aroA* genes of *Escherichia coli* constitute a mixed-function operon, the nucleotide sequence of which is known (Duncan & Coggins, 1986). However, it has been proposed, through the use of a *serC-aroA-lac* fusion plasmid, that the operon is expressed in a cAMP-dependent manner (Lim *et al.*, 1994). The expression of the *serC-aroA-lac* fusion plasmid is significantly elevated in *E. coli* cells grown in a minimal medium with lactose as a unique carbon source. The *serC-aroA* operon may provide a means for co-ordinating the expression of the two genes in response to environmental changes. However, regulation of the *E. coli serC-aroA* operon has not been elucidated in detail, and the physiological significance of the operon is not yet known. This study describes additional evidence in support of the cAMP-dependent expression of the *serC-aroA* operon at a transcriptional level.

Materials and Methods

Materials

Ampicillin, O-nitrophenyl β-D-galactopyranoside (ONPG),

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adenosine 3′,5′-cyclic monophosphate (cAMP), L-glutamic acid, L-glutamic dehydrogenase, NADH, 3-hydroxypyruvate, bovine serum albumin, ficoll, polyvinyl pyrrolidone, and D-glucose were purchased from Sigma Chemical Co. Bacto agar, Bacto yeast extract, and Bacto tryptone were obtained from Difco Laboratories. A Prime-a-Gene Labelling System was supplied by Promega Corporation, and [α-^{35}S] dATP (1,000 Ci/mmol) was obtained from Amersham Corporation. All other reagents were of analytical grade, or the highest quality commercially available.

Bacterial strains and plasmids

All bacterial strains used were derivatives of *E. coli* K-12. Strain MC1061 [*hsdR rglB araD139*Δ(*araABC-leu*) Δ*lacZ74 galU*] and strain CL100 (*cya thi pro his leu lacZ*) were used for measurement of β-galactosidase activity expressed from the *serC-aroA-lac* fusion plasmid. Strain HMS22 (*endA gal thi his rglA rglB*) was used to determine the *serC-aroA* mRNA content after the addition of cAMP.

Plasmid pWH2 carries a *serC-aroA-lac* fusion plasmid (Lim *et al.*, 1994), which contains the regulatory region of the *serC-aroA* operon, the entire coding region of *serC* gene, and the part of *aroA* gene coding the N-terminal amino acids.

Bacterial growth

The *E. coli* strains were routinely grown in Luria-Bertani broth (LB broth; 10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter) or LB solid agar (LB broth plus 1.5% agar). They were also cultured in M9 minimal medium (Na_2HPO_4 7 g, KH_2PO_4 3 g, NaCl 0.5 g, NH_4Cl 1 g, 20% glucose 20 ml, 0.01 M $CaCl_2$ 10 ml, 0.1 M $MgSO_4$ 10 ml per liter) and solid minimal agar (M9 minimal medium plus 1.5% agar) after supplementation with required components. All *E. coli* strains used were cultured at 37℃. Ampicillin (50 μg/ml) was added to media, if necessary.

Preparation of crude extract

Crude extracts were prepared from bacterial cultures grown in LB broth or M9 minimal medium. Cells were harvested and resuspended in 0.1 M phosphate buffer (pH 7.3). The cells were disrupted by sonication, then centrifuged to remove cell debris and membrane-bound enzymes.

Protein concentrations in crude extracts were determined by the method of Lowry *et al.* (1951).

Enzyme assays

β-galactosidase : β-galactosidase activity in crude extracts was determined according to a slight modification of the method of Miller (1972). For high β-galactosidase activity, the absorbance change at 420 nm was directly scanned after the preparation of a reaction mixture. For low β-galactosidase activity, the reaction mixture was incubated for an appropriate time, then the absorbance at 420 nm was measured.

PSAT : The assay for PSAT activity was based on the method described by Duncan and Coggins (1986). The formation of α-ketoglutarate from 3-hydroxypyruvate was coupled to the glutamate dehydrogenase reaction. The assay mixture (1 ml) contained 50 mM Tris-HCl (pH 8.2), 32 mM ammonium acetate, 2 mM glutamate, 0.2 mM NADH, 2.5 mM 3-hydroxypyruvate, and 4 U of glutamate dehydrogenase. The reaction was started by addition of 3-hydroxypyruvate, and the absorbance change at 340 nm was directly recorded.

RNA dot blot hybridization

To quantify the *serC-aroA* mRNA content a dot blot assay was performed as described by Gabi *et al.* (1993) and Kornblum *et al.* (1988). Total RNA was prepared from *E. coli serC*$^+$ *aroA*$^+$ HMS22 cells grown in M9 medium with exogenously added cAMP (0.5~4 mM). Extracted RNA was transferred onto a nitrocellulose filter and prehybridized with Denhardt's solution (Denhardt, 1966). for 4 to 5 h at 57℃. Hybridization was carried out in Denhardt's solution containing approximately 5×10^6 cpm of probe DNA for 12 to 16 h at 57℃. The 500 bp *Cla*I DNA fragment (Lim *et al.*, 1994), which covers a part of the *serC* coding region from the *serC-aroA* operon, was labeled with [α-^{35}S] dATP using a Prime-a-Gene Labelling System (Promega). The filter was washed with two changes of 2 ×SSC (0.15 M NaCl, 0.015 M Na_3-citrate, pH 7.0) and 0.1% SDS for 30 min each at 37℃, then twice with 0.1×SSC and 0.1% SDS for 30 min. The blot was dried and autoradiographed at −80℃ using an intensifying screen. The mRNA content was quantified by liquid scintillation counting after excision of the hybridized portion from the nitrocellulose filter.

Results and Discussion

Effect of exogenously added cAMP

Expression of the *serC-aroA* operon was monitored by measuring β-galactosidase activity in strains carrying plasmid pWH2 (Lim *et al.*, 1994), in which *lacZ* expression is directed by the transcriptional signals of the *serC-aroA* operon as well as the *aroA* translational signals. The effect of exogenously added cAMP on β-galactosidase synthesis in *cya*$^+$ and *crp*$^-$ strain MC1061 cells carrying pWH2, which had grown in LB broth, was examined (Fig. 1). The specific activity of β-galac-

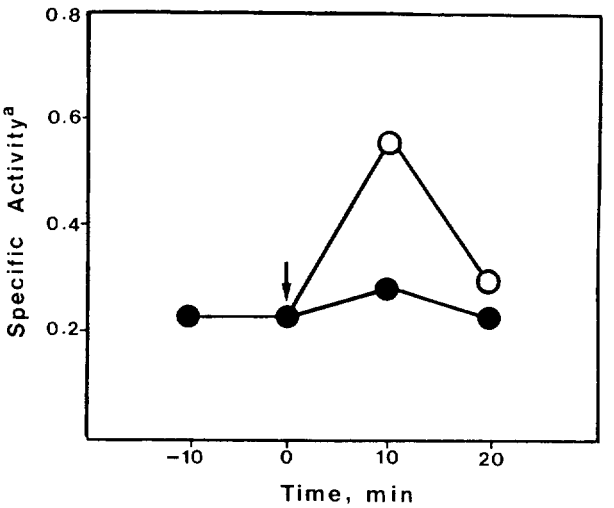

Fig. 1. Effect of exogenous cAMP on the β-galactosidase synthesis in MC1061 carrying a pWH2 grown in LB broth media. During exponential growth, the culture was split (marked by an arrow), and cAMP (1 mM) was added to one culture flask (○–○). Closed circles (●–●) indicate β-galactosidase synthesis in the absence of cAMP. The β-galactosidase activity was determined by the spectrophotometric assay using O-nitrophenyl-β-galactopyranoside (ONPG) as a substrate. Reaction mixture contained 112 mM β-mercaptoethanol, 1 mM $MgCl_2$, 2.27 mM ONPG, and cell extract in 0.1 M phosphate buffer (pH 7.3), and change in absorbance was directly measured at 25℃. [a]OD_{420}/min/mg protein.

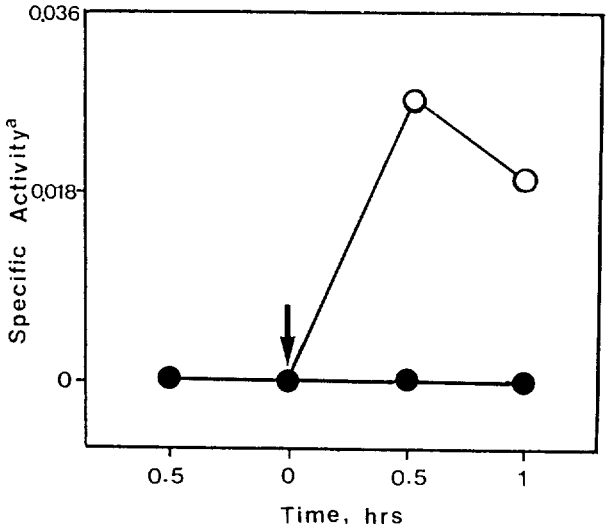

Fig. 2. Effect of the exogenous cAMP on the β-galactosidase synthesis in the *cya* strain CL100/pWH2. The strain was grown in M9 minimal media, and during the exponential growth, the culture was split (marked by an arrow). Open and closed circles indicated β-galactosidase synthesis in the presence (○–○) and absence (●–●) of 1 mM cAMP, respectively. Due to low β-galactosidase activity, the reaction mixture was incubated at 28℃ for an appropriate time before measuring the absorbance at 420 nm. [a]OD_{420}/min/mg protein.

tosidase increased approximately 2.5-fold at 10 min after the addition of exogenous cAMP (1 mM), then decreased at 20 min after addition. This supports the idea that exogenous cAMP induces β-galactosidase synthesis from the *serC-aroA-lac* fusion plasmid. This induction was more evident in *cya*$^-$ strain CL100/pWH2 (Fig. 2). Strain CL100/pWH2, grown in M9 minimal medium, caused an increase in β-galactosidase synthesis at 30 min after the addition of exogenous cAMP. However, the same cells exhibited a low level of β-galactosidase activity in the absence of exogenous cAMP. This distinct effect clearly shows that expression of the *E. coli serC-aroA* operon requires the presence of cAMP. *cya*$^-$ strain CL100 cells grew better in a glucose-minimal medium supplemented with serine and aromatic amino acids (Fig. 3). This situation can be understood since glucose in a minimal medium causes a decrease in cAMP concentration, and the resultant low level of cAMP reduces the expression of the *serC-aroA* operon. Therefore, supplementation of serine and aromatic amino acids helps the growth of *cya*$^-$ cells in a minimal medium.

Expression of the chromosomal *serC* gene

With the use of the *serC-aroA-lac* fusion plasmid the expression of the *serC-aroA* operon was shown to be positively regulated by the cAMP-CRP complex. However, changes in the activity of the SerC or the AroA product were not measured. In the use of the fusion plasmid another negative aspect could be that a multi-copy plasmid was used in the regulation study. To provide convincing evidence of cAMP-dependent expression of the *serC-aroA* operon, the specific activity of PSAT, encoded by the *serC* gene, was measured in *cya*$^+$ *serC*$^-$ *aroA*$^+$ strain HMS22 cells grown under the experimental conditions. Strain HMS22 was grown in M9 minimal medium with either glucose or lactose as the sole carbon source, and the PSAT activity was determined in the cells at the exponential and stationary phases. As shown in Table 1, the PSAT activity of the cells grown in lactose-minimal medium was 1.7-fold higher in the exponential phase and 2.4-fold higher in the stationary phase than that of cells grown in glucose-minimal medium. This agrees with the proposed regulation mechanism for the expression of the serC-aroA operon, since the cAMP level is much higher in lactose-grown cells than in glucose-grown cells (Botsford & Drexler, 1978; Epstein *et al.*, 1975).

The influence of exogenously added cAMP was also examined with *cya*$^-$ *serC*$^+$ *aroA*$^+$ strain CL100 cells. Various amounts of cAMP were added to exponential cultures of CL100, and the PSAT activity was measured at 30 min after addition. The base level of PSAT activity in *cya*$^-$ cells was found to be much lower than the level in *cya*$^+$ cells (Table 1 & Fig. 4). This may

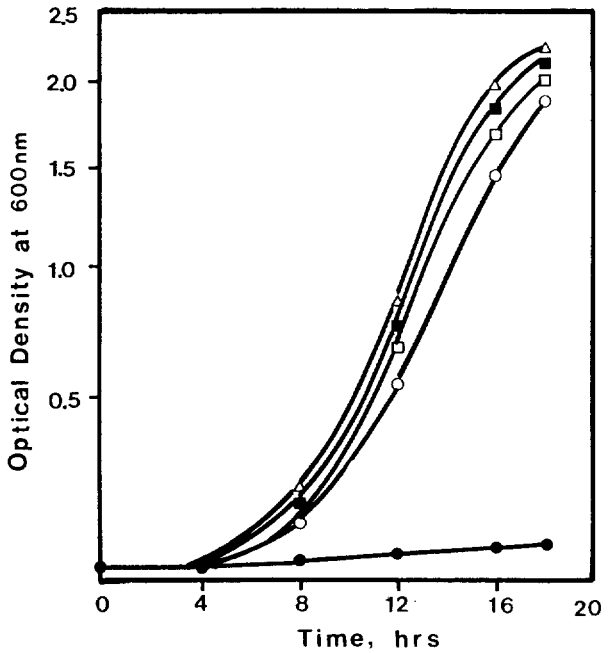

Fig. 3. Growth curves of *E. coli* CL100 in M9 minimal media. ●–●: CL100 in M9 media with lactose; ○–○: CL100 in M9 media with glucose; □–□: CL100 in M9 media with glucose and aromatic amino acids; ■–■: CL100 in M9 media with glucose and serine; △–△: CL100 in M9 media with glucose, serine and aromatic amino acids.

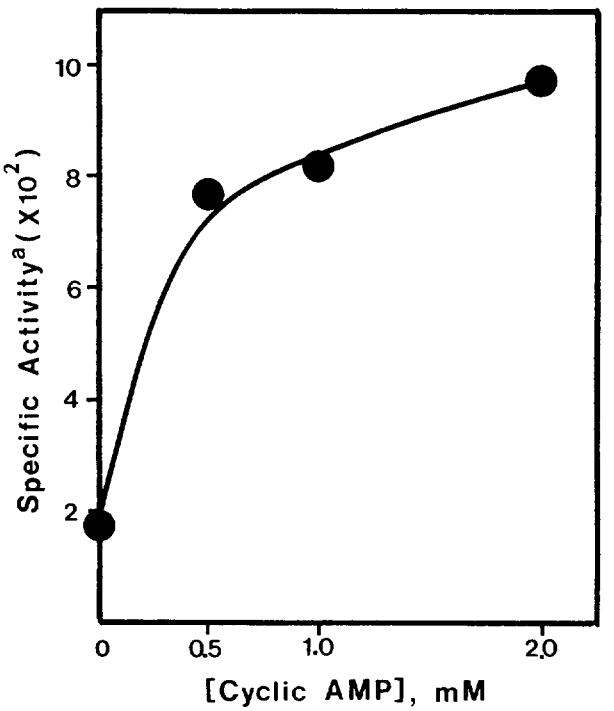

Fig. 4. Effects of exogenous cAMP on the PSAT activity in the *cya*$^-$ strain CL100 grown in M9 minimal medium. During exponential growth, the culture was split and PSAT activity was measured at 30 min after the addition of cAMP. The assay procedure was described in the legend of Table 1. ^{a}OD_{340}/min/mg protein.

Table 1. The measurement of PSAT activity in the extracts prepared from the HMS22 cultures grown in M9 minimal medium with glucose or lactose*

Media	Specific activity^a	
	Exponential phase	Stationary phase
M9/glucose	6.2×10^{-2}	3.1×10^{-2}
M9/lactose	10.5×10^{-2}	7.3×10^{-2}

*Strain HMS22 was grown in M9 minimal medium with either glucose or lactose as the sole carbon source, and the PSAT activity was determined in the cells of exponential and stationary phases. Assay mixture (1 ml) contained: 50 mM Tris-HCl (pH 8.2), 32 mM ammonium acetate, 2 mM glutamate, 0.2 mM NADH, 2.5 mM 3-hydroxypyruvate and 0.1 mg (4 U) of glutamate dehydrogenase (Sigma). The reaction was initiated by the addition of 3-hydroxypyruvate and the absorbance change at 340 nm was measured. ^{a}ΔOD_{340}/min/mg protein.

Table 2. Effects of serine hydroxamate, glyphosate, and Fe^{3+} ion on the production of β-galactosidase from the *serC-aroA-lac* fusion plasmid*

Additions	Relative β-galactosidase activity
Control	100
+1 mM L-serine	114.0
+1 mM L-serine hydroxamate	44.5
+1 mM $FeCl_3$	101.0
+4 mM $FeCl_3$	99.9
+1 mM 2,2′-dipyridyl	93.7

*The Δ*lac* strain MC1061 carrying a *serC-aroA-lac* fusion plasmid pWH2 was grown in M9 minimal meidum. During exponential growth, the culture was split and measured at 30 min after the addition of an appropriate component. The assay procedure was described in the legend of Fig. 1.

be due to the absence of cAMP in the *cya*$^-$ cells. However, the exogenously added cAMP greatly enhanced PSAT activity in the *cya*$^-$ cells (Fig. 4). At cAMP concentrations of 0.5, 1.0, and 2.0 mM in the medium, the specific activities of PSAT were 4.3-, 4.5-, and 5.4-fold higher than the control level before the addition of cAMP. The addition of cAMP apparently caused a great increase in the synthesis of PSAT in *cya*$^-$ cells. This increase confirms that the *E. coli serC-aroA* operon is positively regulated by cAMP.

Effect of serine hydroxamate and Fe^{3+} ions on the expression of the *E. coli serC-aroA* operon

Serine and chorismate, the end products of the two pathways catalyzed by EPSP synthase and PSAT, are precursors of enterochelin. Therefore, the affect of iron

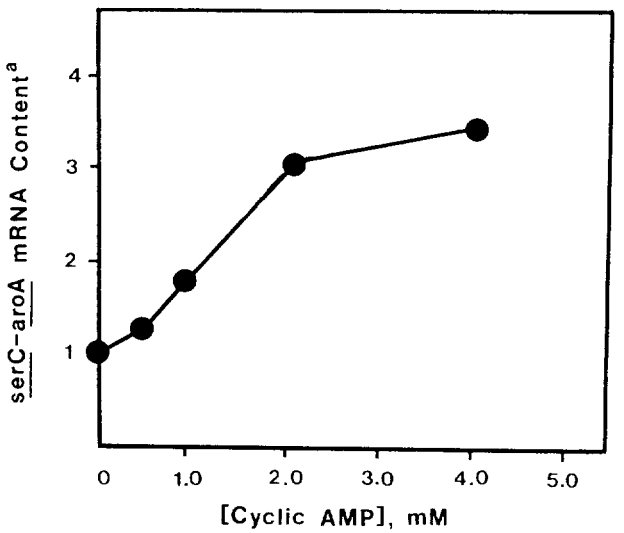

Fig. 5. Concentration effect of exogenous cAMP on the *E. coli serC-aroA* mRNA content. The *serC-aroA* mRNA content was determined by a dot blot assay in the *serC*$^+$ *aroA*$^+$ HMS22 cells. Total RNA was prepared from the same number of cells grown in LB broth in the presence of exogeneously added cAMP (0.5~4 mM). The denatured RNA was fixed onto a nitrocellulose filter and hybridized to the radioactive 500 bp *Cla*I DNA fragment covering the N-terminal part of *serC*-coding region. Quantitation of *serC-aroA* mRNA was by liquid scintillation. [a]The relative values were calculated by considering the *serC-aroA* mRNA content in the cells before the addition of cAMP as 1.

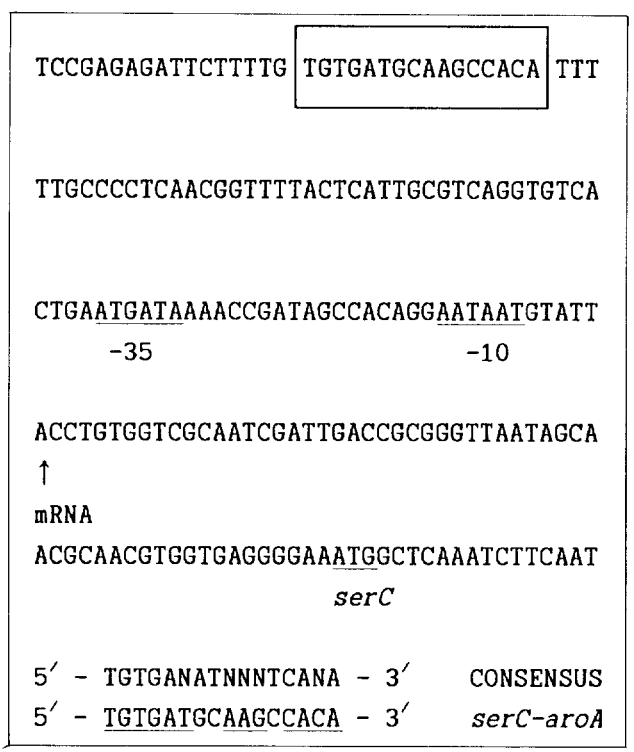

Fig. 6. The nucleotide sequence of the regulatory region of the *E. coli serC-aroA* operon. The site for initiation is indicated by an arrow, and the corresponding -10 and -35 regions for the promoter are underlined. The location of a potential binding site for cAMP-CRP complex is marked by a box. The bottom side shows its resemblance to the consensus sequence.

Consensus sequence	5′ -AAA	TGTGA	NATNNN	TCANA	- 3′
serC-aroA operon	5′ -TTG	TGTGA	TGCAAG	CCACA	- 3′
lac operon	5′ -TAA	TGTGA	GTTAGC	TCACT	- 3′
AMP nucleosidase gene	5′ -TTC	TGTGA	CATACT	ATCGG	- 3′
crp gene	5′ -TAA	TGTGA	CGTCCT	TTGCA	- 3′
araBAD operon	5′ -AAG	TGTGA	CGCCGT	GCAAA	- 3′
gal operon	5′ -AAG	TGTGA	CATGGA	ATAAA	- 3′
cir gene	5′ -AGA	TGTGA	AGCGAT	AACCC	- 3′
cya gene	5′ -AGG	TGTTA	AATTGA	TCACG	- 3′

Fig. 7. A comparison in the various regulatory regions of genes regulated by cAMP-CRP complex. Boxes indicate the site for cAMP-CRP binding.

on the expression of the *serC-aroA* operon was examined. In concentrations of 1 and 4 mM $FeCl_3$ it did not have any effect on the expression of the *serC-aroA-lac* fusion plasmid (Table 2). 2′,2′-dipyridyl, an iron-chelating agent, also had no effect on plasmid expression. L-serine hydroxamate, an analog of L-serine, reduced expression of the *serC-aroA-lac* fusion plasmid, the meaning of which is as yet unknown.

Quantification of the *serC-aroA* mRNA

To investigate the effect of cAMP on the *serC-aroA* operon at the level of transcription, the *serC-aroA* mRNA content was examined. The *serC-aroA* mRNA content was measured by a dot blot assay in *serC*$^+$ *aroA*$^+$ *cya*$^+$ cells grown in a minimal medium with various concentrations of exogenously added cAMP (Fig. 5). The 500 bp *Cla*I DNA fragment used as a radioactive probe carries the N-terminal coding region of the *serC* gene (Duncan & Coggins, 1986). At a cAMP concentration of 2.0 mM in the medium the *serC-aroA* mRNA content increased approximately 3-fold by 30 min after addition. Moreover, the content appeared to increase in a concentration-dependent manner. This result demonstrates that cAMP causes an increase in the *serC-aroA* mRNA content, and that the cAMP effect occurs at the transcriptional level. Furthermore, a sequence found in the regulatory region of the *E. coli serC-aroA* operon (Fig. 6), 5′-TGTGATG-CAAGCCACA-3′, appears to be similar to the cAMP-CRP binding site consensus sequence. This sequence

was compared with the various regulatory regions of genes regulated by the cAMP-CRP complex (Fig. 7).

In *E. coli* cAMP plays a crucial role in gene expression as a function of carbon source. These data suggest that the expression of the *serC-aroA* operon is related to the carbon source in the medium. In other words, syntheses of serine and aromatic amino acids can be affected by cAMP, which would be a rare event. However, the physiological role of cAMP in the *serC-aroA* operon is not yet certain. It may be related to one of the pleiotropic characters of the mixed operon. Further study is required to understand cAMP-dependent expression of the *E. coli* mixed-function *serC-aroA* operon.

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