Transcription of the *Escherichia coli* Adenylate Cyclase Gene Is Negatively Regulated by cAMP-cAMP Receptor Protein*

(Received for publication, February 16, 1984)

Hiroji Aiba

*From the Radioisotope Laboratory, Faculty of Medicine, Kyoto University, Kyoto 606, Japan*

The regulatory region of the *Escherichia coli* cya gene was analyzed by using S1 nuclease mapping and in vitro transcription experiments. The cya gene was transcribed, both *in vivo* and *in vitro*, from one major promoter (P2) and two weak promoters (P1 and P1') that are located about 200 base pairs upstream of P2. The transcription from P2 was specifically inhibited by cAMP-CRP (cAMP receptor protein) *in vitro*. This regulatory mechanism was shown to be physiologically relevant through quantitative analyses of the cya mRNA in intact cells by S1 and dot blot assays. DNase I protection experiments revealed that cAMP-CRP binds to the cya DNA region between +11 and -20, in which a consensus CRP binding sequence is present. Moreover, it was found that CAMP-CRP alters the binding of RNA polymerase to the promoter region, thus inhibiting the transcription of the cya gene.

In both procaryotes and eucaryotes, cAMP controls a number of biological activities as a result of changes in the intracellular concentration of the nucleotide. A well-known example of cAMP-mediated regulation is catabolite repression in bacteria (1-3). Glucose and its analogs lower intracellular cAMP levels, which in turn causes a reduction in the expression of a set of catabolite-sensitive operons such as *lac*, *ara*, and *mal*. In these operons, cAMP and its receptor protein (CRP) act as a positive effector for transcription. On the other hand, it has been shown recently that cAMP-CRP1 represses transcription of another set of genes (4-6). In addition, this complex seems to be involved in modulating transcriptional termination in certain polycistronic operons (7, 8). Thus, cellular reactions under cAMP control and the modes of cAMP action are relatively well-understood (9-12).

However, our knowledge of the regulation of intracellular cAMP levels is very limited. Among the various factors that affect the intracellular cAMP level, the rate of cAMP synthesis seems to be of major importance (3, 9). To clarify the control of cAMP synthesis, it is necessary to study the regulation of adenylate cyclase activity and cya gene expression. Recently the cya genes from *Escherichia coli* (13-16) and from *Salmonella typhimurium* (17) have been cloned and partially characterized. It is now possible to study cya expression at the molecular level.

In this paper, I demonstrate that the cya gene of *E. coli* is transcribed from one major and two minor promoters, with the transcription from the major promoter being negatively regulated by cAMP-CRP both *in vitro* and *in vivo*. Moreover, DNase I footprinting studies indicate that the cAMP-CRP complex binds to a site which overlaps extensively with the RNA polymerase binding site. I conclude that cAMP-CRP is a repressor for the cya transcription.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Preparation of DNA Fragments**—The cya plasmids pCA2 and pCA3 (16) were purified according to the procedure of Birnboim and Doly (18). Plasmid DNAs were digested by appropriate restriction endonucleases and the resulting fragments were electrophoretically fractionated on 6% polyacrylamide gels.

**DNA Sequencing and S1 Mapping**—The nucleotide sequence of the 396-bp EcoRI-BamHI fragment (7, see Fig. 1) was determined by the method of Maxam and Gilbert (19). For the S1 mapping experiment, fragments 1, 3, and 7 (see Fig. 1) were labeled at the 5' ends with 32P and their strands were separated on an 8% acrylamide gel (19). Each single-stranded DNA was hybridized to cellular RNAs and treated with S1 nuclease, and the products were analyzed on an 8% polyacrylamide gel containing 8 M urea. Cellular RNAs were purified from exponentially growing *E. coli* cells in L broth medium. The strains used were pp6 (wild type), pp47 (crp7), and pp47 carrying the crp plasmid pH7 (20). Detailed procedures for S1 mapping were described previously (6, 21).

**In Vitro Transcription**—The reaction mixture consisted of 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 2.5 μg of bovine serum albumin, 1-2 μg of RNA polymerase (a gift from N. Shimamoto, Hiroshima University, Hiroshima, Japan), and 50-100 ng of DNA fragment in a total volume of 27 μl. Where indicated, 10-9 M cAMP, CTP, or 0.1 μg of CRP was added to the reaction mixture. Following incubation for 5 min at 37°C, 3 μl of a ribonuclease mixture containing 1 mM [α-32P]UTP (5-10 μC) and 2 mM each of ATP, CTP, and GTP were added. After 10 min of incubation at 37°C, transcription was terminated by adding 50 μl of phenol, 30 μl of 0.6 M sodium acetate (pH 5.5), 20 mM EDTA, and 100 μg/ml of tRNA. The products were precipitated with ethanol and analyzed on 8% polyacrylamide gels in 8 M urea. CRP was purified from *E. coli* strain pp47 containing the multicopy crp plasmid pH7 (20) by the procedure of Ellen et al. (22).

**RNA Sequencing**—RNAs II and V (see Fig. 4) were synthesized in *in vitro* using fragments 2 and 3 (see Fig. 1), respectively, as templates. The reaction mixture consisted of 20 mM Tris-HCl (pH 8.0), 100 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, 1 mM dithiothreitol, 10 μg of RNA polymerase, 1 μg of DNA fragment, 200 μM ATP, GTP, CTP, and UTP in a total volume of 200 μl. After 30 min of incubation at 37°C, the reaction was terminated by adding 200 μl of phenol. The RNA products were precipitated with ethanol and separated by electrophoresis in a 6% polyacrylamide-8 M urea gel. RNAs II and V were extracted from the gel, treated with calf intestinal phosphatase, and then labeled with [γ-32P]ATP at their 5' ends by T4 polynucleotide kinase. The labeled RNAs were purified again by electrophoresis as above. The 5'-end labeled transcripts were sequenced by enzymatic methods using the RNA sequencing kit (containing RNAases T1, U2, Pho M, and Bacillus cereus) from P-L Biochemicals.

**Dot Blot Hybridization**—A dot blot assay of RNA was carried out essentially according to the procedure of Thomas (23). Total cellular RNAs from *E. coli* strains pp6 and pp47 were incubated in 0.55 M
phenol and precipitated with ethanol. The sample was dissolved in water and then treated with H2O and then formaldehyde and 7.5 mM sodium phosphate buffer (pH 7.0), at 65 °C for 7 min. The reaction mixture was cooled on ice and the denatured RNAs were spotted onto dry nitrocellulose paper which had been treated with H2O and then 20 × SSC. The nitrocellulose paper was baked for 2 h at 80 °C and hybridized with nick-translated DNA probe.

DNase I Footprinting—In the DNase I protection experiments, 50 ng of labeled fragment 6 (see Fig. 1) in 100 µl of transcription buffer (described above) containing 5 mM CaCl2 was incubated for 5 min at 25 °C in the presence and absence of 2 µg of CRP, 10-4 M cAMP, and 15 µg of RNA polymerase. DNase I (Worthington) was added at a concentration of 100 ng/ml and the incubation was continued for 40 s at 25 °C. After adding 25 µl of 1.5 M sodium acetate, and 20 mM EDTA, containing 100 µg/ml of tRNA, the mixture was treated with phenol and precipitated with ethanol. The sample was dissolved in 10 µl of 40% formamide, 5 M urea, 5 mM NaOH, 1 mM EDTA, 0.025% bromphenol blue, and xylene cyanol, and electrophoresed on 8% polyacrylamide-8 M urea gels.

RESULTS

Organization of the cya Promoter—The physical map of the cloned cya gene of E. coli has been established previously (16). The relevant restriction cleavage sites in this cya promoter region are shown in Fig. 1 along with the DNA fragments used in this study. It has been demonstrated that a promoter for the cya gene is located between two BamHI sites and the gene is transcribed from left to right (14-16). DNA sequence studies suggested that the translation of the cya gene starts between two BamHI sites (15, 16) and terminates in the region about 150 bp downstream of HindIII site.2 Amino acid sequence analysis of cya-lacZ hybrid protein revealed that the cya translation starts with an unusual TTG codon at position +155 (24) (see Fig. 2). The precise position of the promoter was determined by S1 mapping and DNA sequencing analysis (16). However, the organization of the cya promoter seems more complex, since another promoter that may direct cya transcription was identified in the 380-bp region between the EcoRI and BamHI sites (14, 15).

Fig. 2 shows the DNA sequence of the region containing cya promoters. The sequence left of BamHI site 1 determined in this work is completely identical with that of Roy et al. (15). To characterize precisely the cya promoters in vivo, 490-bp HindIII fragment 3 32P-labeled at both 5' ends was strand-separated, and an S1 mapping experiment was conducted with each strand. When the 5' end-labeled lower strand (coding strand) was used as a DNA probe, a strong DNA band A was observed along with weak bands B, C, and F (Fig. 3, lanes 2 and 3). Band A is about 120 bases long, corresponding to mRNA originating from the first promoter (P2). The precise transcriptional start point for P2 is 118 nucleotides upstream from the HindIII site (16).

The position of the second promoter was tentatively localized by Roy et al. (15), on the basis of a promoter-like sequence which occurs just upstream of BamHI site 1. However, the length of bands B and C (330-350 bases) implies that the second promoter should be located about 100 bp upstream from BamHI site 1, and may be composed of two overlapping promoters. To confirm this, S1 mapping was repeated using the lower strand of the 380-bp EcoRI-BamHI fragment, which occurs just upstream of BamHI site 1. However, the band B seems to be a run-off transcript, and a much stronger band is seen in the upper strand of the 380-bp EcoRI-BamHI fragment (Fig. 3, lanes 2 and 3), indicating that P2 is the major promoter for cya transcription. This is consistent with the results of Roy et al. (15), who showed in their cya-lac fusion studies that P2 directs the synthesis of five times as much β-galactosidase as P1 and P1'.

In addition, another promoter (P3), which initiates leftward transcription, was also identified in the 380-bp EcoRI-BamHI fragment by Roy and Danchin (14). This seems to be a promoter for an unknown gene which codes for a 40,000-dalton protein (14). The upper strand of the 32P-end-labeled fragment 3 as a DNA probe, I obtained an S1-resistant DNA band F of 165 bases long (Fig. 3, lane 7). When the upper strand of the 32P end-labeled fragment 7 was used as a DNA probe, the protected band G was 290 bases long (Fig. 3, lane 9). These results indicate that Px is located in a region about 80 bp to the left of the BamHI site 1. Inspection of the DNA sequence revealed a leftward Pribnow sequence around -200, accompanying a -35 sequence. Bands F and G were also produced when the lower strands of fragments 3 and 7 were used as DNA probes (lanes 2, 3, and 5), possibly due to contamination of the lower strands by upper strand. Interestingly, Px is located within the cya P1 and P1' regions. Thus, the binding of RNA polymerase to Px could interfere with the transcription from P1 and P1'. This may partly explain why P1 and P1' are weak promoters.

Characterization of the cya Promoters in Vitro—In vitro transcription experiments were carried out using purified DNA fragments containing the cya promoter region. First, the 529-bp BamHI fragment 1, which is truncated at a distance of 416 bases from the P2 start site, was transcribed. As shown in Fig. 4A, lane 1, a major transcript I, of about 420 bases long, is made. The size of this transcript is the same as that of the predicted run-off P2 RNA. When the 629-bp HpaII fragment 2, which contains all four promoters identified in vivo, is transcribed, the main transcript II is about 310 bases long, corresponding in size exactly to the expected run-off P2.
Negative Regulation of cya Transcription by cAMP-CRP

Fig. 2. Location of the cya promoters and DNA-protein contact sites. Boxes, Pribnow sequences. Double lines above or below the sequence, -35 sequences. Horizontal arrows, transcriptional start sites. Brackets, the regions protected by cAMP-CRP and RNA polymerase from DNase I attack. Vertical arrows, sites where DNase I digestion was enhanced in the presence of protein factors. Dashed lines, sequences homologous with the canonical CRP-binding sequence (12, 27).

transcript (Fig. 4A, lane 3). In addition, two other RNAs (III and IV) are synthesized from the fragment 2 (lane 3). The length of RNAs III and IV was estimated to be about 500 and 120 bases, respectively. This is well-explained by assuming that RNA III is the transcript from P1 and P1' and RNA IV is that from P2.

Likewise, the 490-bp HinfI fragment 3 was transcribed to give RNA V of 120 bases, RNA VI of 170 bases, and RNA VI1 of 250 bases, which correspond to the predicted P2 and P1' RNAs, respectively (Fig. 4B, lane 4).

To confirm the origin of the transcripts, the 5' ends of RNA II and RNA V were analyzed by RNA sequencing technique. The sequence of RNA V (Fig. 5) and RNA II (data not shown) was shown to be ppp-GUUUUAGA-, which is identical to the predicted sequence of P2 RNA. Although the sequence of the other RNAs was not determined, the size and direction of these transcripts suggest that the four promoters identified in vivo, the three cya promoters (P1, P1', and P2), and the promoter (Px) for the unknown gene, are also functional in vitro.

Specific Inhibition of P2 Transcription by cAMP-CRP

Based on the observation that cAMP synthesis is strongly enhanced in cells with a defective CRP, it has been suggested that cya expression is negatively regulated by CAMP-CRP at the transcriptional level (25, 26). However, to date no direct evidence for this negative control has been obtained. To test this hypothesis, the effect of CAMP-CRP on cya transcription was studied by using the in vitro system described above. Examination of the transcription of the 629-bp HpaII fragment 2 revealed that CAMP-CRP inhibits the transcription from P2. As shown in Fig. 6A, lane 3, when CRP along with cAMP was added to the reaction mixture, the amount of P2 transcript (RNA I1) was dramatically reduced, while that of the Px transcript (RNA IV) was unaffected. Neither CRP (lane 2) nor cAMP (data not shown) alone had any effect on the P2 transcription. The effect of CAMP-CRP on the transcription of the 490-bp HinfI fragment 3 was also examined. The transcription from P2 (RNA V) was again shown to be repressed by cAMP-CRP (Fig. 6B, lane 2), while the transcription from Px (RNA VI) was not affected by this complex. The transcription from P1 and P1' (RNA VII in Fig. 4A, lane 3, RNA VII in Fig. 4B, lane 2) seemed to be unaffected by cAMP-CRP. The data clearly show that cAMP-CRP inhibits
specifically the transcription from the major cya promoter, P2, in vitro.

Evidence for Negative Regulation of cya Transcription by cAMP-CRP in Vivo—If cAMP-CRP negatively regulates cya transcription in intact cells, one may expect that cells with a defective CRP should produce more cya mRNA than wild type cells. To examine the effect of CAMP-CRP on cya transcription in vivo, one may expect that cells with a defective CRP in vivo.

In Fig. 8A, lanes 2 and 3, the protective effect of RNA of crp− cells against S1 attack of the cya DNA probe was about five times greater than that of crp+ cells. This finding corresponds well to that of the dot blot assay, indicating that the cya transcription is negatively regulated by CAMP-CRP in vivo.

As a second assay, an S1 digestion experiment was performed to measure the levels of cya RNA in crp+ and crp− strains. The 5′ end-labeled lower strand of the 529-bp BamHI fragment 1 was used as a DNA probe to determine the P2 transcript in vivo. Fragment 1 contains the sequence corresponding to the first 416 nucleotides of P2 RNA which includes the translational start site along with 88 codons for the amino-terminal region of the adenylate cyclase (24). The DNA probe was hybridized to cellular RNAs prepared from exponentially growing E. coli cells, treated with S1 nuclease, and the products were analyzed on an 8% polyacrylamide-8 M urea gel. The radioactivity of an S1-resistant DNA band of the first 416 bases is a function of the RNA concentration when an excess single-stranded DNA probe is used (data not shown).

As shown in Fig. 8A, lanes 2 and 3, the protective effect of cya RNA of crp− cells against S1 attack of the cya DNA probe was about five times greater than that of crp+ cells. This finding corresponds well to that of the dot blot assay, indicating that the cya transcription is negatively regulated by CAMP-CRP in vivo.

In Fig. 4, A in vitro transcription of restriction fragments containing cya regulatory region. A, transcripts produced from 100 ng of fragment 1 (lane 2) and 100 ng of fragment 2 (lane 3). Lane 1, DNA size markers. B, transcripts produced from 50 ng of fragment 3 (lane 2), 50 ng of fragment 4 (lane 2), and 100 ng of fragment 5 (lane 4). Lane 1, DNA size markers. Transcription was performed as described under “Experimental Procedures” using 1 μg (A, lanes 2 and 3; B, lanes 2 and 3) or 2 μg (B, lane 4) of RNA polymerase. The transcripts were fractionated on 8% polyacrylamide-8 M urea gels. The numbers on the left represent base lengths of DNA markers. The Roman numerals on the right indicate RNA transcripts.
Negative Regulation of cya Transcription by CAMP-CRP

FIG. 5. Nucleotide sequence of the 5' end of RNA V. RNA V 32P-labeled at its 5' end was partially digested by RNase T1 (lane 1, G specific), RNase Phy M (lane 2, A + U specific), RNase U2 (lane 3, A specific), and RNase B. cereus (lane 4, C + U specific). Lane 5, sequence ladder obtained by partial alkaline digestion. The digests were fractionated on a 25% polyacrylamide-8 M urea gel.

negatively regulates the cya transcription from P2 in vivo as well as in vitro.

Binding of CAMP-CRP to the cya Regulatory Region—In the previous work (16), we demonstrated that there exist two sites containing the consensus CRP binding sequence 5'-AA-TGTGA--T---TCA-ATT-3' (12, 27) in the cya regulatory region. One (site A) is located around the P2 transcriptional start site, from +4 to -18, and the other (site B) is between +124 and +107. This and the specific inhibition by cya transcription mentioned above strongly suggest that CAMP-CRP could interact with the putative CRP site(s) in the cya gene. To test this, I performed a DNase I protection experiment using a restriction fragment containing the cya regulatory region. Fig. 9 shows the effects of CAMP-CRP and/or RNA polymerase on the digestion by DNase I of the 275-bp BamHI-RsaI fragment 6 32P-labeled either at its RsaI 5' end (lower strand, Fig. 9A) or its BamHI 5' end (upper strand, Fig. 9B). It was found that CAMP-CRP protected from DNAse I attack the region between -17 and +11 on the upper strand (Fig. 9B, lane 4) and that between -20 and +5 on the lower strand (Fig. 9A, lane 4), while CRP alone did not affect the digestion pattern (lane 3). The region protected by CAMP-CRP corresponds well to the predicted CRP site A mentioned above. On the other hand, no significant interaction with CAMP-CRP was observed in the putative CRP site B (data not shown). As expected, RNA polymerase protected a longer DNA region corresponding to the cya promoter in both strands (lane 5). Therefore, the DNA segment protected

FIG. 6. Inhibition of cya transcription by CAMP-CRP. A, effect of CAMP-CRP on the transcription of fragment 2. Lane 1, RNA polymerase alone; lane 2, 0.1 µg of CRP added; lane 3, 0.1 µg of CRP and 10 µM cAMP added. B, effect of CAMP-CRP on the transcription of fragment 3. Lane 1, RNA polymerase alone; lane 2, 0.1 µg of CRP and 10 µM cAMP added. Transcription was carried out using 1 µg of RNA polymerase and 100 ng of DNA fragment as described under "Experimental Procedures."

FIG. 7. Dot blot assay of cya RNA in intact cells. Denatured cellular RNAs (0.3, 1.5, and 7.5 µg) from pp6 (wild type) and pp47 (crp') were spotted onto the nitrocellulose paper and hybridized with 50 ng (107 cpm) of the nick-translated BamHI-HindIII fragment 8 (A) or HinfI-HindIII fragment 9 (B).
Negative Regulation of cya Transcription by cAMP-CRP

FIG. 8. Determination of P2 and Px RNAs by S1 digestion assay. Lower strand of fragment 1 (A) and upper strand of fragment 7 (B). 32P-labeled at their 5' ends, were used as DNA probes to determine the levels of cya P2 RNA and Px RNA, respectively. 32P-labeled DNA probe (50,000 cpm) was hybridized to 100 µg of RNAs extracted from exponentially growing wild type pp6 (lane 2) or crp-pp47 (lane 3) or pp47 carrying the crp plasmid pHA7 (lane 4) and treated with 10 units of S1 nuclease for 10 min at 37 °C. Products were analyzed on 8% polyacrylamide-8 M urea gels. Lane 1 is a DNA probe without S1 treatment. Arrows represent DNA bands protected from S1 digestion by P2 and Px RNAs.

by cAMP-CRP extensively overlaps with the RNA polymerase binding site.

To examine whether cAMP-CRP prevents RNA polymerase binding to the cya promoter, the partial DNase I reaction was performed in the presence of both cAMP-CRP and RNA polymerase. Lane 6, in Fig. 9, A and B, shows the result of adding cAMP-CRP first, then RNA polymerase followed by performing the DNase I reaction. An experiment in the reverse order (RNA polymerase then cAMP-CRP) was also done and the same protection pattern was obtained. Thus, cAMP-CRP alters the protection pattern by RNA polymerase, although polymerase can still bind to the promoter region in the presence of cAMP-CRP. These results indicate that cAMP-CRP inhibits the cya transcription by interfering with the functional binding of RNA polymerase to the promoter. The location and the function of the cAMP-CRP binding site match well with those of the typical operator site (28, 29).

DISCUSSION

The most striking feature concerning the regulation of cAMP synthesis is that mutants lacking CRP have greatly increased levels of cAMP (25, 26, 30, 31). This immediately suggests that CRP negatively regulates either the activity or the synthesis of adenylate cyclase. In fact, more adenylate cyclase activity was found in crp- strains than in crp+ strains even in the presence of CAMP-CRP. These results indicate that CAMP-CRP interacts with the unique site, containing a consensus CRP binding sequence (12, 27), which overlaps with the RNA polymerase-promoter interaction is altered in the presence of CAMP-CRP. It is apparent that CAMP-CRP inhibits the cya transcription by preventing the functional binding of RNA polymerase to the promoter. This molecular mechanism is essentially the same as that of the inhibition of the lac transcription by the lac repressor which also alters the RNA polymerase binding (28).

Bankaitis and Bassford reported about a 2-fold increase in the inhibitory effect of cAMP-CRP on the cya transcription in their fusion studies (32). The present results are basically compatible with this observation. However, those investigators concluded that cAMP-CRP does not play a significant role in transcriptional or translational regulation of the adenylate cyclase gene, since the repressive effect was weak. On the other hand, Roy et al. (15) did not observe any repressive effect of cAMP-CRP in their studies using multicopy fusion plasmids. The reason for this may be that the cya transcription is moderately repressed in wild type cells, since this regulation is a type of autogenous control. If cAMP-CRP strongly represses cya expression as it does in vitro, the level of cAMP will finally decrease and cya transcription may increase resulting in more messages. Actually, I repeated the S1 digestion and the dot blot assays to measure the levels of cya RNA in crp+ and crp- strains by using cellular RNAs prepared under different growth conditions. I found that the repression ratio fluctuates between 2 and 5 depending on growth conditions. Therefore, if the levels of either cAMP or CRP decrease under some conditions, the repressive effect of cAMP-CRP on cya transcription should be reduced. This may explain why the previous gene fusion studies observed very weak (32) or no (15) repressive effect of cAMP-CRP, since the experimental conditions used in fusion studies are different from those in the present studies. It is also possible that the fusions
alter the regulation of the cyclase promoter in some unknown manner.

Although the data presented here confirmed the existence of a negative control of cya transcription by cAMP-CRP, they do not exclude the possibility that the activity of adenylate cyclase is negatively regulated by CAMP-CRP. Recent progress in the purification of E. coli adenylate cyclase (34) may stimulate further studies to elucidate the regulatory aspects of this important enzyme.

A major role of cAMP-CRP is considered to be the transcriptional activation of a number of operons. In the past few years, however, evidence has accumulated indicating that the complex acts as a negative effector for transcription of other genes. It has been shown that one of the two overlapping promoters of the gal operon is inhibited by cAMP-CRP (4, 21). Similarly, CRP inhibits ompA transcription in vitro (5).

In these cases, CRP binds to the −35 region of the respective promoter. More recently, I have shown that the cAMP-CRP complex inhibits the transcription of the cya gene by binding to a unique site located downstream from the transcriptional start point (6). The cya gene is an additional example of which transcription is negatively regulated by cAMP-CRP. Although the location of the CRP binding sites in all four genes described above are different with respect to the transcriptional start site, they all contain a DNA site homologous to the canonical CRP binding sequence 5′-AA-TGTGA--T---TCA-ATT-3′ (12, 27) found in operons where cAMP-CRP acts as a positive effector. It is of interest to note that the CRP binding sequence of the cya is on the coding strand, as in the other three genes where CRP acts as a repressor.

In addition, some of phosphate-regulated genes have been shown through gene fusion studies to be negatively controlled by cAMP-CRP (35). Moreover, the analysis of polypeptides synthesized under various growth conditions has demonstrated that cAMP-CRP reduces the synthesis of nearly as many gene products, including outer membrane protein and glutamine synthetase, as it regulates positively (36, 37). It is possible that the negative control by cAMP-CRP of these genes may occur mostly at the transcriptional level as in the case of cya and crp. cAMP is also known to control a variety of biological activities such as cell morphology and replication of certain plasmids (9). These effects could be a consequence of turning on and/or off a group of cAMP-modulated genes. Detailed analysis of such genes may contribute to our understanding of the molecular basis of these complex biological phenomena.

Acknowledgments—I am grateful to N. Shimamoto for RNA polymerase, to H. Ohkubo and D. Mrozek for reading the manuscript, and to M. Tabata for her typing assistance.

REFERENCES
1. Pastan, I., and Perlman, R. (1970) Science (Wash. D. C.) 169, 339–344
2. Rickenberg, H. V. (1974) Annu. Rev. Microbiol. 28, 355–365
3. Pastan, I., and Adhya, S. (1976) Bacteriol. Rev. 40, 527–551
4. Musso, R. E., Di Lauro, R., Adhya, S., and de Crombrugghe, B. (1977) Cell 12, 847–854
5. Movva, R. N., Green, P., Nakamura, K., and Inouye, M. (1981) FEBS Lett. 128, 186–190
6. Aiba, H. (1988) Cell 52, 141–149
7. Ullmann, A., Joseph, E., and Danchin, A. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3194–3197
8. Guidi-Rontani, C., Danchin, A., and Ullmann, A. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5798–5801
9. Botsford, J. L. (1981) Microbiol. Rev. 45, 620–642
10. Adhya, S., and Garges, S. (1982) Cell 29, 287–289
11. Ullmann, A., and Danchin, A. (1983) Adv. Cyclic Nucleotide Res. 15, 1–53
12. de Crombrugghe, B., Bushy, S., and Buc, H. (1984) Science (Wash. D. C.) 224, 831–838
Negative Regulation of cya Transcription by cAMP-CRP

13. Roy, A., and Danchin, A. (1981) Biochimie 63, 719–722
14. Roy, A., and Danchin, A. (1982) Mol. Gen. Genet. 188, 465–471
15. Roy, A., Haziza, C., and Danchin, A. (1983) EMBO J. 2, 791–797
16. Aiba, H., Kawamukai, M., and Ishihama, A. (1983) Nucleic Acids Res. 11, 3451–3465
17. Wang, J. Y. J., Clegg, D. O., and Koshland, D. E., Jr. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 4684–4688
18. Birnboim, H. C., and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523
19. Maxam, A. M., and Gilbert, W. (1980) Methods Enzymol. 65, 499–560
20. Aiba, H., Fujimoto, S., and Ozaki, N. (1982) Nucleic Acids Res. 10, 1345–1361
21. Aiba, H., Adhya, S., and de Crombrugghe, B. (1981) J. Biol. Chem. 256, 11905–11910
22. Eilen, E., Pampeno, C., and Krakow, J. S. (1978) Biochemistry 17, 2469–2473
23. Thomas, P. S. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5291–5295
24. Danchin, A., Guiso, N., Roy, A., and Ullmann, A. (1984) J. Mol. Biol. 175, 403–408
25. Botsford, J. L., and Drexler, M. (1978) Mol. Gen. Genet. 165, 47–56
26. Majerfeld, I. H., Miller, D., Spitz, E., and Rickenberg, H. V. (1981) Mol. Gen. Genet. 181, 470–475
27. Ebright, R. H., Cossart, P., Gicquel-Sanzey, B., and Beckwith, J. (1984) Nature (Lond.) 311, 232–235
28. Schmitz, A., and Galas, D. J. (1979) Nucleic Acids Res. 6, 111–117
29. Gussalus, R. P., and Yanofsky, C. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7117–7121
30. Potter, K., Chaloner-Larsson, G., and Yamazaki, H. (1974) Biochem. Biophys. Res. Commun. 57, 379–385
31. Wayne, P. K., and Rosen, O. M. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 1436–1440
32. Bankaitis, V. A., and Bassford, P. J., Jr. (1982) J. Bacteriol. 151, 1346–1357
33. Joseph, E., Bernsley, C., Guiso, N., and Ullmann, A. (1982) Mol. Gen. Genet. 185, 262–268
34. Yang, J. K., and Epstein, W. (1983) J. Biol. Chem. 258, 3756–3758
35. Wanner, B. L. (1982) J. Mol. Biol. 166, 283–308
36. Prusiner, S., Miller, R. E., and Valentine, R. C. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 2922–2926
37. Mallick, U., and Herrlich, P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5520–5523