Regulation of Lac Transcription in Escherichia coli by Cyclic Adenosine 3',5'-Monophosphate

STUDIES WITH DEOXYRIBONUCLEIC ACID-RIBONUCLEIC ACID HYBRIDIZATION AND HYBRIDIZATION COMPETITION

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SUMMARY

Cyclic adenosine 3',5'-monophosphate (cyclic AMP) is required for the synthesis of lac mRNA and β-galactosidase in Escherichia coli and cell-free extracts. Competition hybridization experiments, described in this report, show that the concentrations of lac mRNA in E. coli are controlled by cyclic AMP, as well as by inducer. Both transient and permanent repression by glucose affect lac mRNA production and are reversed by cyclic AMP. In an adenyl cyclase-deficient mutant strain, lac mRNA is not synthesized until the cells are supplied with cyclic AMP. A mutant strain lacking a recently described "cyclic AMP receptor protein" does not make lac mRNA despite exogenous cyclic AMP. We conclude that cyclic AMP, cyclic AMP receptor protein, an intact promoter locus, and other unidentified factor or factors are necessary for efficient transcription of the lac operon.

Cyclic adenosine 3',5'-monophosphate is currently believed to be required for the synthesis of inducible enzymes in bacteria and for the cell-free synthesis of β-galactosidase (1-3). In recent reports we have described DNA-RNA hybridization experiments demonstrating that cyclic AMP stimulates the synthesis of lac mRNA, both in growing Escherichia coli and in cell-free extracts (4, 5). In this paper, we present a relatively simple competition hybridization technique for the measurement of lac mRNA concentrations in unlabeled cells. Results with this assay demonstrate regulation of lac mRNA levels by cyclic AMP, thus confirming predictions made in our earlier studies. In addition, we examine lac transcription in mutant strains deficient in adenyl cyclase and in a recently described "cyclic AMP receptor protein" (6, 7). Results with these strains support the notion that cyclic AMP and a normal cyclic AMP receptor protein as well as an intact lac promoter gene are required for lac mRNA synthesis (8-10). We also show that cyclic AMP regulates lac transcription in wild type strains under conditions of permanent as well as transient glucose repression.

METHODS AND MATERIALS

Bacteria, Media, and Chemicals—The bacterial strains employed in this report are listed in Table I. Growth media and chemical sources have been previously described (4).

Enzyme assay—β-Galactosidase activity was determined as described by Pardee, Jacob, and Monod (11). One unit of enzyme is the amount which hydrolyzes 1 nmole of o-nitrophenyl-β-D-galactoside per min at 28°.

Phage DNA—Phage were obtained by heat induction of growing cultures of E. coli RV usually labeled with 14C-thymidine for several hours after induction. After cell lysis, extracted phage were purified by banding in cesium chloride density gradients and deproteinized by phenol treatments as previously described (4).

Bacterial RNA—Labeled and unlabeled RNA were extracted from growing bacterial cultures by the method of Okamoto, Sugino, and Nomura (12). Preparations were either dialyzed against 4-fold concentrated SSC (SSC, 0.15 M NaCl, 0.015 M sodium citrate) or lyophilized to dryness and resuspended in 4-fold concentrated SSC prior to hybridization.

Hybridization Methods—λ80 DNA and λ80lac DNA were immobilized on Schleicher and Schuell type B6 nitrocellulose filters according to the procedure of Gillespie and Spiegelman (13). Filters 8 mm in diameter, containing 0.03 to 0.3 μg of DNA, were punched out of 27-mm filters loaded with 0.015 M sodium citrate or lyophilized to dryness and resuspended in 4-fold concentrated SSC prior to hybridization.

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Calculations—For competition hybridization, the radioac-
tivity of a saturating amount of labeled lac mRNA annealed to λ80dIac DNA in the absence of competing unlabeled RNA was determined in each experiment, and experimental points were calculated as a percentage of this control value. 

Competition curves were then plotted, or the results were transformed to linear functions using the formula derived by Stubbs and Hall (14). This conversion permits an assessment of the relative concentrations of unlabeled lac mRNA in the tested preparations on the basis of comparative slopes of the linear functions. Data are plotted according to the formula \( Q_0 - Q_c/Q_0 = f.C/tL \), where \( Q_0 \) represents counts per min of lac hybridized in the absence of unlabeled competitor RNA, \( Q_c \) equals counts per min of lac annealed in the presence of competitor RNA at concentration \( C \), \( t_L \) is the amount of labeled RNA used in each reaction mixture (and is therefore a constant in each experiment) and \( f \) is the proportion, by weight, of lac mRNA in an unlabeled RNA preparation. When \( Q_0 - Q_c/Q_0 \) is plotted against the concentration of test RNA (\( C \)), the resulting slope (\( f.C/tL \)) is a relative measure of the concentration of lac mRNA in each RNA preparation.

In competition experiments, the filters contained about 10 cpm of \(^3H\)-labeled DNA at the settings employed for double isotope counting. \(^3H\) values were corrected for the amount of DNA on each filter. This procedure did not materially affect results, which comprised an average of three or four filters, but did reduce the standard error of the mean. In each experiment, the counts per min hybridizable to λ80 DNA were assumed to represent the non-lac background interactions and were subtracted from experimental points.

For direct hybridization experiments, \(^3H\)-labeled RNA preparations of known specific activity were incubated with control and lac-containing DNA filters. The percentage of \(^3H\)-labeled RNA annealing with each type of filter was calculated, and the "percentage of difference" was determined, as previously described (4). This value is a measure of accumulation of labeled lac mRNA during the period of labeling, and is principally dependent upon the rate of lac mRNA synthesis (4).

RESULTS

As the first step in development of a competition hybridization assay, it was necessary to determine the amount of \(^3H\)-RNA required to saturate available lac DNA sites. Increasing amounts of labeled RNA extracted from cells induced for \(\beta\)-galactosidase synthesis were hybridized with filters containing 0.03 \(\mu\)g of \(\lambda\)80dIac DNA (Fig. 1). The marked decrease in the slope of the curve in Fig. 1 indicates that saturation is approached between 5 and 10 \(\mu\)g of RNA. In competition experiments shown here, generally 6 to 8 \(\mu\)g of \(^3H\)-labeled RNA are incubated with each filter.

The sensitivity of the competition assay was evaluated by preparing unlabeled RNA from wild type cultures grown in the presence of increasing concentrations of inducer; we then compared the capacity of these RNA preparations to compete with labeled lac mRNA for lac DNA sites. As shown in Fig. 2A, increasing concentrations of IPTG induce increasing amounts of \(\beta\)-galactosidase synthesis with 5 \(\times\) \(10^{-4}\) M IPTG. The cells were labeled with \(^3H\)-uridine (1 mCi per 5 \(\times\) \(10^{10}\) cells) from 7 to 10 min after the addition of IPTG; specific activity of the \(^3H\)-labeled RNA was 6.18 \(\times\) \(10^{6}\) cpm per \(\mu\)g. --- , based on two experimental points, estimates the amount of "background" annealing to \(\lambda\)80 DNA filters.

![Fig. 1. DNA saturation curve. Filters containing 0.03 \(\mu\)g of \(\lambda\)80dIac DNA were incubated with increasing amounts of \(^3H\)-labeled RNA extracted from a culture of \(E. coli\) 3000 induced for \(\beta\)-galactosidase synthesis with 5 \(\times\) \(10^{-4}\) M IPTG. The cells were labeled with \(^3H\)-uridine (1 mCi per 5 \(\times\) \(10^{10}\) cells) from 7 to 10 min after the addition of IPTG; specific activity of the \(^3H\)-labeled RNA was 6.18 \(\times\) \(10^{6}\) cpm per \(\mu\)g. --- , based on two experimental points, estimates the amount of "background" annealing to \(\lambda\)80 DNA filters.](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2196093/)

**TABLE I**

| Strain          | Characteristics                             | Source or Description |
|-----------------|---------------------------------------------|-----------------------|
| \(E. coli\) K-12 3000 | Wild type, lac \(l^p^o^z^y^a^+\) \(mel^-\) | Provided by E. Steers |
| \(E. coli\) W4032 | lac deletion, \(pro^r\) \(met^-\)           | Developed by E. Lederberg, acquired through S. Falkow |
| \(E. coli\) 5336 | lac\(^+\), adenyl cyclase\(^+\)             | (6)                   |
| \(E. coli\) 5333 | lac\(^+\), cyclic AMP receptor protein\(^-\) | (7)                   |
| \(E. coli RV\) | lac deletion, double lysogen for \(\lambda\)80 and \(\lambda\)80dIac | Developed by E. Siger, acquired through M. Yarmolinsky |
| \(E. coli\) 1103 | lac\(^+\), Enz \(^+\), F\(^-\)              | F. Fox (4)            |
| \(E. coli\) X7700 | lac\(^-\), \(pro^-\) \(L1_{401}\) \(pro^-\) | (8, 10)               |
| \(E. coli\) X8047 | lac\(^-\), Enz \(^+\), F\(^-\)              | (8, 10)               |
Comparison of direct and competition hybridization with *E. coli* 3000

For direct hybridization experiments, growing cultures of the wild type strain were treated with $5 \times 10^{-4} \text{ M}$ IPTG alone, with $25 \text{ mM}$ glucose, or with $5 \text{ mM}$ cyclic AMP and glucose. After 5 min, samples were removed for $\beta$-galactosidase assay and 2 min later the cultures were pulse labeled for 120 sec with $^3H$-uridine (1 mCi per $10^8$ cells). The $^3H$-labeled RNA was extracted, specific activities determined, and 2 $\mu$g of each preparation hybridized with filters containing 0.15 $\mu$g of $\phi 80d\lambda$ac DNA, in the presence of 60 $\mu$g of unlabeled RNA from *E. coli* W4029. The competition hybridization experiments are described in the legend to Fig. 3; samples were removed for $\beta$-galactosidase assay after 5 min of incubation. To facilitate comparison of enzyme and $lac$ mRNA determinations in these experiments, values are presented relative to an induced level of 1.00.

![Table II](http://www.jbc.org)

| Additions | Direct hybridization | Competition hybridization |
|-----------|---------------------|--------------------------|
|           | Specific activity $\times 10^3$ | Relative $\%$ difference | Relative enzyme level | Relative slope | Relative enzyme level |
| IPTG      | 1.23                | 1.00                      | 1.00                   | 1.00          | 1.00                   |
| IPTG, glucose | 1.05              | 0.00                      | 0.15                   | 0.10          | 0.13                   |
| IPTG, cyclic AMP | 1.83            | 0.79                      | 0.85                   | 0.80          | 0.85                   |

Because competition hybridization compares unlabeled RNA preparations, it is not subject to variations in rates of $^3H$-uridine incorporation observed particularly upon addition of glucose to wild type cells. In our previously reported direct hybridization experiments, we largely circumvented this difficulty by studying a mutant strain (*E. coli* 1103), unable to metabolize glucose because of deficiency of Enzyme I of the $p$-enolpyruvate transferase system but still susceptible to transient repression (4, 15). In Table II, we present data from direct hybridization experiments with *E. coli* 3000. Although glucose substantially increases over-all $^3H$-uridine incorporation, the difference between percentage of input counts per min hybridized to $\lambda$80d$\lambda$ac DNA and that hybridized to $\lambda$80 DNA correlates well both with enzyme synthesis and with $lac$ mRNA concentrations, as determined by competition hybridization.

In our earlier experiments with *E. coli* 1103, it was not, of course, possible to measure $lac$ mRNA synthesis during permanent (or catabolite) repression by glucose, since this strain does not metabolize glucose. We have shown elsewhere that high concentrations of cyclic AMP ($3 \times 10^{-3}$ m) can reverse catabolite repression of $\beta$-galactosidase synthesis (8). We have now determined $lac$ mRNA production in wild type cells grown in glucose for several generations (Fig. 4). Induced cells make 2 to 3 times as much $lac$ mRNA or $\beta$-galactosidase when supplied with adequate amounts of cyclic AMP. As expected, very little enzyme or message is detected in the absence of inducer. Because all three cultures are glucose grown, the extent of labeling by $^3H$-uridine is similar. These results indicate that the effect of cyclic AMP in catabolite, as well as transient, repression is exerted upon transcriptional events.

We felt that a more stringent test of the hypothesis that cyclic AMP is required for $lac$ mRNA synthesis could be performed with the adenyl cyclase-deficient strain, *E. coli* 5336. This strain contains no detectable adenyl cyclase or cyclic AMP and is unable to metabolize a variety of carbohydrates until supplied with exogenous cyclic AMP. As shown in Fig. 5, when this

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Fig. 2. *lac* mRNA concentration as a function of inducer concentration. Cultures of *E. coli* 3000 growing in Medium A, 0.5% glycerol, and thiamine, 5 $\mu$g per ml, were induced with three different concentrations of IPTG for 12.5 min; a fourth culture, to which no inducer was added, served as a control. At the end of the induction period, the RNA was extracted from each cell culture. Increasing amounts of each unlabeled RNA species were then incubated with filters containing 0.05 $\mu$g of $\lambda$80d$\lambda$ac DNA in the presence of 6.2 $\mu$g of $^3H$-labeled RNA from an induced culture. The specific activity of the $^3H$-labeled RNA was $3 \times 10^{6}$ cpm per $\mu$g, and 417 cpm (specific) were annealed at saturation in the absence of competing RNA. The percentage of specific counts per min competed by the unlabeled RNA preparations is plotted in Panel A. In Panel B, these results are plotted linearly according to the transforming formula described in the text. *$\beta$-Galactosidase synthesis in each culture is depicted in Panel C.* No inducer, $\square$; IPTG 2.5 $\times 10^{-4}$ M, $\triangle$; IPTG 5.0 $\times 10^{-4}$ M, $\bullet$; IPTG 5.0 $\times 10^{-4}$ M, $\otimes$.

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[Table II](http://www.jbc.org)
CATABOLITE REPRESSION

Fig. 4. Lac mRNA synthesis during catabolite repression and cyclic AMP treatment. Cultures of E. coli 3300 were grown in Medium A supplemented with 0.5% glucose and 5 μg per ml of thiamine. Two cultures were induced for β-galactosidase synthesis with 5 × 10⁻⁴ M IPTG. One of these cultures was simultaneously treated with 5 mM cyclic AMP. A third culture served as an uninduced control. After 8 min of induction, samples were removed for β-galactosidase assay and 2 min later the cultures were labeled for 20 sec with [³H]-uridine (1 mCi per 10⁶ cells). Samples (2 μg) of [³H]-labeled RNA extracted from each culture were hybridized with filters loaded with 0.15 μg of Xh80 or Xh80dZac DNA in the presence of 60 μg of unlabeled RNA from the lac deletion strain. Striped bars indicate “percentage of difference” hybridized, stippled bars show β-galactosidase content. Since the uninduced cells have negligible levels of β-galactosidase, the amount present at 8 min is a measure of the rate of enzyme synthesis.

When strain is induced with IPTG, it produces no more enzyme than lac mRNA than is found in the uninduced culture. When cyclic AMP is added, production of both returns to the range seen in induced wild type strains.

A similar result is obtained with the competition hybridization assay (Fig. 6). Unlabeled RNA from a culture induced in the absence of cyclic AMP competes poorly for lac sites compared with the competing ability of RNA from a cyclic AMP-treated culture. Therefore, both direct and competition hybridization experiments with the mutant strain indicate that cyclic AMP is required for the synthesis of lac mRNA.

The availability of an adenyl cyclase-deficient strain also affords an opportunity to test the hypothesis that cyclic AMP prevents premature termination of lac transcription (16). This proposal implicates that small species of RNA homologous to lac DNA might be found in cultures of E. coli 5336 induced in the absence of cyclic AMP. Cultures exposed to cyclic AMP as well as inducer would be expected to contain lac mRNA ranging in size up to 30 S polycistronic material. As described in detail in the legend to Fig. 7, [³H]-labeled RNA from uninduced, induced, and cyclic AMP-treated induced cultures were sedimented in sucrose gradients and fractions were pooled for hybridization studies. About half of the large amount of lac mRNA in the cyclic AMP-treated cells sediments between 14 S and 26 S; the remainder is divided equally between material sedimenting beyond 26 S and that sedimenting from 6 S to 14 S. Presumably, the largest material represents intact, polycistronic lac mRNA, and the other species represent lac mRNA in the various stages of synthesis and degradation expected in view of its short half-life (4). For each class of RNA, however, the same low levels of lac-specific material are found in the uninduced culture and in the induced culture not receiving cyclic AMP. (As discussed in our previous report, this small amount of RNA hybridizing preferentially to λ80dla DNA probably consists primarily of the transcription product of the i gene (4, 17). A very small amount of material transcribed from genes adjacent to and cotransduced with lac and a minor amount of RNA transcribed from structural lac genes may also be present.) This result suggests that the effect of cyclic AMP is not to prevent abortive transcription, but to augment frequency of transcription. Experiments performed with lac promoter mutants and with inhibitors of transcription support this suggestion (8–10, 18).

A recent report from this laboratory (7) describes the isolation of a cyclic AMP receptor protein which binds cyclic AMP and is believed to be required for cyclic AMP action both in vivo and in vitro. Mutant strains lacking a normal cyclic AMP receptor protein have been isolated. These strains are unable to metabolize a large number of carbohydrate, including lactose,
but contain high adenyl cyclase activity and high levels of cyclic AMP. Moreover, they do not respond to additional exogenous cyclic AMP. We measured lac mRNA synthesis in one of these strains (E. coli 5333) and found minimal production of lac mRNA (or β-galactosidase) in response to inducer, even when extra cyclic AMP was supplied. These data are presented in Table III and compared with results of experiments with wild type cells. These results demonstrate that efficient lac transcription cannot occur in strains lacking normal cyclic AMP receptor protein, despite the presence of a normal lac operon, a gratuitous inducer, and cyclic AMP.

Previously reported experiments from this laboratory and others (8-10) have indicated that an intact lac-promoter gene is also required for normal regulation of lac enzyme synthesis by cyclic AMP and glucose. Lac-promoter mutants are generally inducible, but they synthesize much less enzyme than their parent strains (2 to 5%) and are less sensitive (or insensitive) to the effects of glucose or cyclic AMP upon enzyme synthesis. Contesse, Crépin, and Gros (19) have demonstrated by DNA-RNA hybridization that strains carrying a lac promoter mutation make much less lac mRNA during induction than do wild type strains. We have confirmed this finding, using E. coli X8047. This strain contains the L1 deletion, extending from the latter part of the i gene through the p locus (10). It therefore synthesizes lac enzymes constitutively, but even in the presence of inducer makes only 2% as much enzyme as its parent strain X7700. Lac mRNA synthesis is also markedly impaired; the percentage of pulse labeled RNA found to hybridize preferentially to lac DNA was found to be 0.036%, thus differing minimally from the 0.025% of uninduced wild type RNA capable of hybridizing to lac DNA. Cyclic AMP in concentrations up to 30 mM did not affect enzyme synthesis in glucose-grown cells and had no significant effect upon lac mRNA production, although both enzyme and lac mRNA production were augmented 2- to 3-fold by cyclic AMP in the parent strain grown under similar conditions. However, small alterations in the very low rate of lac transcription seen in the mutant strain could have occurred and been undetectable with our assay. Thus we cannot definitely exclude an effect by cyclic AMP upon lac transcription in promoter mutants, although it seems unlikely in view of correlations between lac mRNA and enzyme production seen under other conditions of cyclic AMP control.
TABLE III
Lac mRNA synthesis in mutant deficient in cyclic AMP receptor protein

| Strain | IPTG | Cyclic AMP | β-Galactosidase | Lac mRNA |
|--------|------|------------|----------------|---------|
|        |      | units/ml   | % Difference   |          |
| 5333   | −    | 0.00       | 0.025          |         |
|        | +    | 0.02       | 0.037          |         |
|        | +    | 0.02       | 0.039          |         |
|        | +    | 0.02       | 0.041          |         |
| 3000   | −    | 0.00       | 0.025          |         |
|        | +    | 3.60       | 0.832          |         |

**DISCUSSION**

Our development of a competition hybridization method for measurement of lac mRNA was stimulated by the reports by Stubbs and Hall (14) of a competition assay for mRNA specific for the tryptophan operon. Because H-RNA from a constitutive tryptophan strain hybridized almost half as well with control DNA as with tryptophan operon-containing DNA, these authors were obliged to purify labeled tryptophan mRNA for their assay. Initially we employed a parallel technique for the isolation of labeled lac mRNA. Although we were able to obtain such material in highly purified form, it proved to be relatively rapidly degraded during storage. Since H-RNA from a strain induced for β-galactosidase hybridizes only to a small extent with control DNA (3 to 10% of the radioactivity annealing to lac-containing DNA), we found that reproducible, significant, and sensitive competition hybridization could be performed using unfractionated labeled RNA from an induced culture. As we have shown in this report, the capacity of unlabeled RNA to compete with labeled material for lac DNA sites depends upon the concentration of inducer and the presence of cyclic AMP in the culture from which the RNA is extracted. These results therefore confirm previous findings that cyclic AMP as well as inducer regulates lac transcription.

Because the competition hybridization assay measures concentration of lac mRNA, rather than the rate of lac mRNA accumulation detected by direct hybridization, it is possible, as illustrated by Stubbs and Hall (14) for tryptophan mRNA, to estimate the number of lac mRNA molecules per cell in a given culture. In the saturation experiment shown in Fig. 1, the filters contain 0.03 µg of λ80dlac DNA. The number of phage DNA copies can be calculated by multiplying this amount of phage DNA (3 × 10^8 g) by Avogadro’s number (approximately 6 × 10^23) and dividing the product by the molecular weight of the phage genome (3 × 10^8 daltons). The result, 6 × 10^8 copies, provides the number of lac operons on each filter. At saturation, when presumably 6 × 10^8 copies of lac mRNA are annealed to an equal number of lac DNA copies, about 5000 cpm of lac mRNA are hybridized. When 0.5 µg of the same H-labeled RNA preparation from an induced culture is hybridized to excess λ80dlac DNA, about 2000 cpm are annealed. Assuming lac hybridization to be virtually complete under these conditions, 1 µg of such RNA contains about 5 × 10^6 copies of lac mRNA. With our extraction method, we obtain approximately 20 mg of RNA from 10^12 cells, or about 2 × 10^8 µg of RNA from each cell. Therefore, we estimate that the average cell in an induced culture contains about 10 copies of lac mRNA. Most cells in uninduced cultures or in cultures unable to synthesize or utilize cyclic AMP have no copies. When cyclic AMP levels are transiently lowered by glucose, cells contain about 1 copy per cell. Since the actual amount of RNA per cell may be somewhat higher than our estimate and since lac hybridization may not be more than two-thirds complete under conditions of DNA excess, it is possible that these calculations account for as little as half the actual number of lac mRNA copies. However, the minimum value of 10 copies per cell for an induced culture is comparable to the minimum of 8 copies of tryptophan mRNA per derepressed cell as determined by Stubbs and Hall (14) in a similar fashion.

In our previous report of regulation of lac mRNA synthesis by cyclic AMP, we used direct hybridization methods which measure accumulation of labeled lac mRNA (4). To show that accumulation was not being affected by changes in rates of lac mRNA degradation, we measured the decay of labeled lac mRNA. This half-life proved to be short (60 to 90 sec) and unaffected by cyclic AMP. Hence we assumed that cyclic AMP augments accumulation of lac mRNA by increasing its synthesis, and we predicted that lac mRNA concentrations would vary in proportion to the rates of synthesis. As shown by the data in Table II, this prediction has now been confirmed by competition hybridization experiments.

Our first report of hybridization studies of cyclic AMP control of lac transcription demonstrated the effect of cyclic AMP only during the severe transient repression observed in the first several minutes after addition of glucose to a growing culture. We have shown elsewhere that cyclic AMP also reverses the permanent, milder form of repression (catabolite repression) of inducible enzyme synthesis caused by prolonged growth on rich media (8). In this paper, we report that reversal of catabolite repression by cyclic AMP is manifest by stimulation of lac mRNA as well as β-galactosidase synthesis.

Further evidence in support of the hypothesis that cyclic AMP is required for lac transcription is provided by hybridization experiments with two recently isolated mutant strains. Induced cultures of E. coli 5336, which lacks adenyl cyclase, appear to synthesize no more lac mRNA (or β-galactosidase) than uninduced cells unless supplied with exogenous cyclic AMP. Moreover, the contention that cyclic AMP prevents premature termination of lac transcription seems improbable in view of the absence of small or large pieces of lac mRNA in cultures not supplied with cyclic AMP, although RNA fragments of less than 10 to 15 nucleotides would not be detected by our assay. The second mutant strain, E. coli 5333, lacks a normal cyclic AMP receptor protein and therefore cannot bind cyclic AMP or synthesize the inducible enzymes dependent upon cyclic AMP. Moreover, cell-free extracts of this strain synthesized β-galactosidase at a low rate unless supplied with cyclic AMP receptor protein from a wild type strain (7). As expected,
the strain deficient in the cyclic AMP receptor protein makes very little lac mRNA in the presence of inducer, even when high levels of cyclic AMP are supplied.

There have been reports of a stimulatory effect of cyclic AMP and a repressive effect of glucose upon translation of lac mRNA into enzyme (20, 21). Although we have been unable to detect translational effects of cyclic AMP in control of the Zac operon, our experiments with the tryptophanase operon, translational effects of cyclic AMP in control of the Zac operon, and a repressive effect of glucose upon translation of Zac mRNA levels of cyclic AMP are supplied.

In attempting to determine whether cyclic AMP acts directly upon transcription, we have previously shown that cyclic AMP augments lac mRNA synthesis in cells in which enzyme production has been halted by the withdrawal of an essential amino acid (4). Moreover, in recent experiments with several antibiotics which block protein synthesis (including chloramphenicol, streptomycin, spectinomycin, and puromycin), responsiveness to cyclic AMP remains in the absence of detectable enzyme synthesis.8 These experiments, however, also favor the notion that lac transcription and translation are tightly coupled processes, since all the antibiotics thus far tested, excepting puromycin, severely inhibit lac mRNA accumulation without decreasing total RNA synthesis. Nevertheless, ribosomal movement along lac mRNA may occur under such conditions, even without complete enzyme synthesis, and provide adequate translational machinery to serve as an agency through which cyclic AMP could affect the slowed transcriptional events (24).

Recent results from our studies of lac transcription in a cell-free system, however, indicate that a stimulatory effect of cyclic AMP can be produced in reaction mixtures containing only lac DNA and a RNA polymerase-containing extract from ribosomes (25). These experiments, and other experiments in which stimulation of in vitro lac transcription by cyclic AMP is preserved despite interference with protein synthesis, suggest that the transcriptional effect of cyclic AMP is direct and not merely mediated through some translational event. Experiments which examine the actions of cyclic AMP upon translation of natural lac mRNA added to a cell-free β-galactosidase-synthesizing system are needed to resolve the question of translational regulation by cyclic AMP.

The possibility that transcriptional control by cyclic AMP might be exerted through translational events raised the further possibility that stimulation of lac mRNA production by inducer might result from repressor-operator interactions at a translational level. Since lac repressor binds to double stranded but not single stranded lac DNA, we reasoned that a preliminary test of the hypothesis would be to look for double stranded lac RNA (26). Pulse labeled RNA preparations from induced and uninduced cultures were digested with T1 and pancreatic ribonuclease. The 1 to 1.5% of the original trichloracetic acid-precipitable material remaining was then heat denatured and hybridized. No lac-specific material was found in either the induced or uninduced preparation, although the sensitivity of the assay was sufficient to detect sequences as short as 15 nucleotides.

Our results suggest at present that several ingredients are required for control of lac transcription by cyclic AMP in E. coli: adenylyl cyclase, cyclic AMP receptor protein, DNA-dependent RNA polymerase, and lac operon DNA containing a normal lac promoter locus. However, we have been unable to demonstrate stimulation of lac transcription by cyclic AMP in simplified reaction mixtures containing cyclic AMP receptor protein, RNA polymerase, and λMd0lac DNA.4 Perhaps other as yet unidentified factors will be implicated in the regulation of transcription by cyclic AMP.

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