CONTROL OF EXPRESSION OF GENETIC INFORMATION
IN E. COLI BY CYCLIC AMP

By

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ABSTRACT

Cyclic AMP is required for the expression of a large variety of dispensable gene functions in E. coli. Cyclic AMP acts at the gene level to permit transcription of genes under its control. To do this cyclic AMP first combines with a cyclic AMP receptor protein and the cAMP-CRP complex alters the DNA so that RNA polymerase can bind in a form ready to initiate transcription. The positive function of cyclic AMP and CRP should be contrasted with the action of the lac and gal repressors whose presence prevents gene transcription. It is only when cyclic AMP and CRP are present and the repressor is neutralized by a specific inducer that transcription of the lac and gal genes can occur.

The excess genetic information carried by organisms permits them to adapt in a limited way to hostile environments. It is to the organism’s advantage to keep these non-essential genes in a quiet state, where when needed they can be rapidly activated. The question at hand is, what mechanisms does the bacterium E. coli employ to switch on and off genes and thereby change its properties in a major way?

E. coli contains genes which code for the synthesis of the enzymes of lactose metabolism. When grown on lactose the organism makes large amounts of β-galactosidase, the enzyme which hydrolyzes lactose to galactose and glucose. However, when the same organism is grown on glucose, only barely detectable
amounts of β-galactosidase are made. On the basis of genetic analysis of *E. coli* containing regulatory mutations in the genes of lactose metabolism (the lac operon), Jacob & Monod proposed that the lac genes were under the control of a large repressor molecule. The repressor acted to prevent transcription of the lac genes (*Jacob & Monod* 1961). Later Gilbert & Muller-Hill isolated the lac repressor, showed that the lac repressor was a protein and demonstrated that it bound to a regulatory site (operator) near the beginning of the lac operon (*Gilbert & Muller-Hill* 1967). Compounds derived from lactose combine with the lac repressor and remove it from the operator site. (Figure 1). The compound most commonly employed in such studies is isopropylthiogalactoside (IPTG), a non-metabolizable lactose analogue.

![Diagram](image)

Fig. 1.

The lac operon and the site of repressor action.

Yet even when *E. coli* are exposed to lactose or IPTG, they cannot synthesize β-galactosidase unless they also have adequate levels of cyclic AMP. In *E. coli* cyclic AMP levels are low when the cells are growing on glucose; the low cyclic AMP levels account for the inability of *E. coli* to synthesize normal amounts of β-galactosidase when confronted with glucose and lactose together (*Pastan & Perlman* 1970). The actions of cyclic AMP can more easily be studied in mutants which have lost the capacity to make cyclic AMP due to a defective adenylate cyclase (*Perlman & Pastan* 1969). Some of the proteins whose synthesis is defective in such mutants are shown in Table 1. Even the gross structure of the organism is altered for such mutants are unable to synthesize flagellae (*Yokota & Gotts* 1970). When *Salmonella typhimurium*, a close relation of *E. coli*, is infected with the bacteriophage P22, the cells lyse, rather than permit the phage to enter into the lysogenic state (*Hong et al.* 1971). How does cyclic AMP control these processes?

We have investigated in detail the action of cyclic AMP on the lac and the galactose (gal) operons. In intact cells, cyclic AMP stimulates the state of synthesis of lac or gal RNA when the cells are exposed to lactose or galactose respectively (*Varmus et al.* 1970; *Miller et al.* 1971).
How then is RNA synthesis stimulated? The ultimate solution to such a question can only come by investigating cyclic AMP’s action in cell-free systems. Such studies have been performed in our own laboratory and also by J. Beckwith, G. Zubay and coworkers.

To study gene transcription DNA containing lac or gal genes in high concentration is needed. Fortunately bacterial viruses which transduce the lac or gal operons are available for such studies. Up to 5% of the DNA of such phage can be lac DNA and perhaps 2–3% gal DNA. When E. coli RNA polymerase is incubated with nucleoside triphosphates and either λh80dlac DNA or λpgal DNA, the 80 and λgenes are transcribed, but the lac and gal genes are not. Even if cyclic AMP is added lac or gal RNA is not made. Apparently an additional factor is needed for cyclic AMP to stimulate lac or gal transcription. E. coli contains a cyclic AMP binding protein. This protein has now been purified to homogeneity (Anderson et al. 1971) and when added to the transcription system together with cyclic AMP, it stimulates lac or gal RNA synthesis (de Crombrugghe et al. 1971; Nissley et al. 1971). In our laboratory this protein has been called CRP or cyclic AMP receptor, it is also known as CAP (catabolite gene activator protein) (Zubay et al. 1971).

CRP is a very basic protein: its isoelectric point is pH 9.1 and it has a very low affinity for DNA. However, when cyclic AMP combines with CRP, the cAMP-CRP complex has a very high affinity for DNA (Pastan et al. 1971; Riggs et al. 1971). Unexpectedly, the complex binds to all types of DNA, most of which do not have genes subject to cAMP-CRP control. The non-specificity of binding is very puzzling and needs further investigation.

Apparently as a consequence of the binding of CRP to DNA, the lac or gal

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promoter is altered so that RNA polymerase can now bind tightly and be ready to initiate transcription (de Crombrugghe et al. 1971; Nissley et al. 1971). This all occurs in the absence of nucleoside triphosphates. Transcription begins when nucleoside triphosphates are added. We know that the RNA polymerase is tightly bound to DNA, because it becomes resistant to inactivation by rifampicin, an antibiotic that rapidly inactivates free RNA polymerase. A model of how cAMP and CRP act is shown in Figure 2.

With a normal lac operon the requirement for cyclic AMP and CRP is almost absolute. Very little lac RNA is made in their absence. However, the promoter region of the lac operon can be altered by mutations which obliterate its requirement for cyclic AMP and CRP (Eron & Block 1971). The development of a system that would transcribe lac DNA permitted us to test the hypothesis that the lac repressor acted by preventing lac transcription. In studies of lac transcription using only purified components, RNA polymerase, λh80dlac DNA, cyclic AMP and CRP, we found that lac repressor was able to prevent lac transcription. Further, the action of repressor was prevented by the addition of the inducer IPTG (de Crombrugghe et al. 1971). Does lac repressor act by preventing RNA polymerase from binding to the lac promoter or by preventing movement of RNA polymerase past the operator? To answer this we first bound RNA polymerase to its promoter binding site and then added repressor. We found that the repressor would still work; no lac RNA was made. It still seemed

![Diagram of cAMP-CRP interaction](image)

**Fig. 2.**
possible that the repressor was displacing RNA polymerase. To examine this, we removed the repressor by adding IPTG and found that lac transcription then occurred (Chen et al. 1971). This result leads us to conclude that RNA polymerase and lac repressor bind at independent sites. Recently we have been able to detect and partially purify the gal repressor (Parks et al. 1971). We are currently studying its action on transcription.

REFERENCES


DISCUSSION

Bourgeois: The Lac repressor and the RNA polymerase have a preference for AT-rich DNAs. Have you compared the binding of CAP to DNAs differing largely in their AT content?

Pastan: We have done quantitative competition experiments, using equal concentrations of DAT or calf thymus DNA. The competition is not very different, maybe no more than 2 fold. We have been looking at a variety of synthetic DNA analogues and I cannot precisely remember the differences, there are some. Both l strand and h strand, lambda pgel DNA compete equally. One thing that I meant to mention was that when
we had done experiments in which we made radioactive cyclic AMP receptor protein by labelling cells with \([1^{14}C]\) amino acids and isolating the protein, this protein would bind in the same non-specific way. We then were able to control the filter binding assay, and we were shocked to find that only 50% of the radioactive protein will stick to nitrocellulose filters. So the filter binding assay can have difficulties that one is not appreciative of.

Bourgeois: It is true that the retention efficiency is not 100% but is characteristic of each protein and constant in standardized conditions?

Pastan: It is a very basic protein, and therefore we thought it would stick tightly to nitrocellulose.

Paul: Have you looked for, and if so, have you found any mutants which involve glucose repression of other loci but not of the lac locus?

Pastan: I don't quite know how to answer that question...

Paul: The point I was getting at was that such mutants might lack a binding locus for CAP in the promoter region.

Pastan: I would like to emphasize that all the studies that we have done suggest that there is no interaction between cyclic receptor protein and RNA polymerase, but that each reacts with DNA, probably at sites that are close together. Various people have looked for polymerase mutants and then asked, do they show normal glucose repressions? Do they respond normally to cyclic AMP? These have in the main been rifampicin resistant mutants. In general, these mutants behave reasonably well to cyclic AMP. So one has not yet found a mutant with a polymerase that is modified.

Paul: It is not a polymerase mutant I am thinking about, it is a promoter mutant, one which may permit binding of polymerase but not regulation by CAP.

Pastan: There is a mutant that has been isolated in Beckwith's laboratory. It is really a double mutant. They started with one mutation that turns the promoter off and then obtained a second mutation within the promoter that turned it back on again. This double mutant makes \(\beta\)-galactosidase. It is inducible, but it is not under cyclic AMP control.

Chambon: In one of your slides you measured the effect of \(\phi\). I think that it has a very striking effect. Would you comment on that?

Pastan: We have studied the action of \(\phi\) on the galactose operon where we get this read-through phenomenon. If we have the operator and promoter here (drawing), then E.T. and K are here. When we do not have \(\phi\) present, polymerase appears to transcribe past K into \(\lambda\) genes. If \(\phi\) is present, there is a cessation of transcription somewhere near the end of K. I am not sure whether it is within the galactose operon at the end of K or within the \(\lambda\) gene just to the left of K. Recently, Dr. de Crombrugghe has been studying insertions in E, which have polar effects in vivo. These insertions appeared to have stop signals that are \(\phi\) sensitive. So the system is sensitive to and one can use it to undertake studies of the \(\phi\) effect.

Erlanger: Does your non-specific binding require cyclic AMP?

Pastan: Yes, I had one slide which shows binding at any variety of condition of pH, salt and anything you might use to decrease non-specific interaction. We have usually
done binding under conditions where transcription is maximally stimulated; it is usually 0.1 to 0.15 molar KCl, 3 millimolar magnesium, pH 7.8. Under these conditions, the binding is completely cyclic AMP dependent. If one lowers the ionic strength or changes the pH, one can start to get non-specific binding.

_Erlanger:_ You mentioned that you use antibody to cyclic AMP for various things. I was wondering if you ever looked at CRP-bound cyclic AMP. When bound to this protein, was it still available for binding antibody? How does it bind to the cell?

_Pastan:_ We have not done that experiment.

_Bourgeois: Smith & Sadler (1971) observed that many 0° mutations also have concomitant promoter effects, i.e. affect the maximum level of lac enzyme synthesis. What is your interpretation of those promoter effects?

_Pastan:_ I think a simpler interpretation is that mutations in the promoter can affect operator function and operator mutations can affect promoter function, and in some way their functions overlap. In some studies we tried to study competition between binding of RNA polymerase and lac repressor using a promoter mutant, a so-called up promoter mutant. We found, with the mutant DNA, that there was partial competition for binding, whereas with the normal lac DNA, there seems to be no competition. That makes me distrust reasoning from mutants. It tells me that the operator and promoter are very close together and that many a linear model of DNA, such as we tend to draw, may be incorrect.

_Dixon:_ What is the specificity for cyclic AMP? Are there any derivatives of cyclic AMP that activate transcription?

_Pastan:_ The only active one is tubericidin cyclic phosphate. Cyclic GMP inhibits binding and inhibits transcription. We have a variety of analogues that will inhibit binding and inhibit transcription. We also have some analogues that will inhibit transcription but not inhibit the binding of cyclic AMP to the cyclic AMP binding protein significantly. This leads us to the idea that there might be two sites on the binding protein. One may be an active site, one may be a regulatory site. The specificity is much higher than the specificity in animal systems where N butyryl compounds, for example, are active.

_Dixon:_ If you have your CAP protein plus cyclic AMP bound to DNA, have you tried the experiment of treating the complex with phosphodiesterase to see if the cyclic AMP is available for cleavage, and if so, does the CAP protein come off?

_Pastan:_ We haven't done that sort of experiment. We have prepared a lot of antibodies to the cyclic AMP receptor protein. If you add cyclic AMP, cyclic AMP receptor protein and λ p gal rDNA, then add antibody before polymerase, you never make _gal_ mRNA. With one antibody preparation, we can still block after RNA polymerase is added. With other antibody preparations, we cannot.

_Mach:_ Does the polymerase displace the receptor protein once it binds to the DNA?

_Pastan:_ We don't know the answer to that because we can't detect specific binding of the cyclic AMP receptor protein, so we cannot ask for displacement. I would very much like to know the answer to that. There actually may be a cycle in which the cyclic receptor protein comes off the DNA, perhaps modified. We have some data that suggest that it could be modified, because during purification, frequency after phospho-
cellulose it is inactivated. It will not bind cyclic AMP and will not stimulate transcrip-
tion. If you simply are patient enough, and let it sit a few days, it first regains cyclic AMP binding activity; then, still later, it will stimulate transcription. That is not always true, but it is commonly observed.

Mach: In cases where you have binding of the non-specific DNA, does that increase the non-specific binding of polymerase to those DNAs?

Pastan: We don't know that, but it doesn't stimulate transcription, except specifically for gal or lac; recently we have studied chloramphenicol acetylate, another gene which is under cyclic AMP control.

Baulieu: In the experiments which you have done, is CAP protein or the complex able to bind to single-stranded DNA?

Pastan: Single-stranded DNA competes in the competition as well as double-stranded DNA. If you use single-stranded DNA as a template for transcription, you do not get any stimulation by cyclic AMP and CRP.

Baulieu: I was not clear about your different binding sites for cyclic AMP. Have you measured how many binding sites there are per subunit?

Pastan: When you measure the number of binding sites, you are limited by the amount of protein you have and the dissociation constants of the sites you are measuring. In the experiments we initially did, we got a few nice points that extrapolated to one binding site with a dissociation constant of $1 \times 10^{-5}$ molar. Now that we had seen these other results, we have a feeling that there might be a second site with a lower affinity. We would need more protein to see this second site.

Baulieu: Would you speak a little about the possibility of such a system playing a role in eukaryotic cells.

Pastan: Nobody understands how cyclic AMP controls gene expression in animal cells. The model of cyclic AMP action that has been well worked out is for phosphorylase activation; this involves phosphorylation. I think that is a plausible mechanism for turning on transcription. There are no data against it, but certainly no one has been able to show that phosphorylating proteins in eukaryotics are obligatory for stimulating transcription. There is one experiment published by Varrone et al. (1972), which shows that cyclic AMP can stimulate transcription of liver DNA with RNA polymerase from rat liver and a special protein fraction. The data could be interpreted as indicating that kinase might not be necessary. Perhaps cyclic AMP is the steroid of bacteria, and steroids don't seem to work through phosphorylation.

Chambon: Does the presence of CRP change the temperature which is required in order to get rifampcin resistant RNA synthesis?

Pastan: The temperature dependence to form a rifampcin resistant complex which would make λ RNA is exactly the same as that which would make lac mRNA. The half life of the complex measured at 37° is about 90 seconds. As far as we can tell by these criteria, the systems are identical.

Erlanger: Just one comment dealing with your results with antibody. One can get deceptive results with antisera when you are looking for inhibition. There frequently is something in antisera that can inhibit an experimental system. Even globulin fractions and antibody purified by specific precipitation might introduce artefacts.
You have to be very careful, especially if you look for inhibition. The only foolproof control to run if you do get inhibition is the supernatant of an antibody preparation that was treated with specific antigen to remove specific antibody. Even in this case, it is preferable that the antigen be in insoluble form.

Pastan: That is possible. I think it is unlikely. We partially purified the antibody by ammonium sulphate precipitation and dialysis, our control is serum from a normal animal; our antiserum did not affect total RNA synthesis, only the cyclic AMP-CRP dependent increment. So I don't think that is our problem. I just recognize the fact that different antibodies have different affinities for proteins and recognize different sites. That is a non-immunologist's interpretation.

References: