THE EFFECT OF CATABOLITE REPRESSION AND OF CYCLIC 3';5' ADENOSINE MONOPHOSPHATE ON THE TRANSLATION OF THE LACTOSE MESSENGER RNA IN ESCHERICHIA COLI

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Cyclic 3';5' adenosine monophosphate (cAMP) has been suggested as playing a role in the regulation of the synthesis of β-galactosidase and some other enzymes and that catabolite repression resulted from depletion of the intracellular cAMP (1,2,3).

From studies on the effect of cAMP on β-galactosidase synthesis in intact cells (4,5) and in a cell-free system (6) it has been concluded that cAMP acts on the synthesis of the lac-mRNA. On the other hand, in the case of the tryptophanase system, cAMP has been reported to operate at the translation level (7). Moreover, in a recent study on catabolite repression of β-galactosidase synthesis (8) it has been shown that the step which is interfered with by catabolite repression is the translation of the lac-mRNA. If cAMP reverses catabolite repression by acting at the same site, the later finding contradicts the view that cAMP acts at the transcription level. Our results suggest that cAMP reverses catabolite repression of β-galactosidase synthesis by releasing the blockage caused in the translation process.

Materials and Methods: Isopropyl-β-D-thiogalactoside (IPTG) and cyclic 3';5' adenosine monophosphate (cAMP) were purchased from Sigma, o-nitrophenyl-β-D-galactoside (ONPG) from Calbiochem, thiamine from California Corporation, proflavin from Edward Gurr and ^{14}C-leucine (50 mC/mMole) from Radiochemical Centre, Amersham.

The strain used in this study was E. coli K-12 Hfr. H, requiring thiamine. It was grown in a medium composed of: 17.7 g $Na_2HPO_4.12H_2O$, 3.0 g KH_2PO_4, 1.0 g NH_4Cl, 0.13 g $MgSO_4.7H_2O$, 1.0 g casein hydrolysate and 1 mg thiamine per liter. Glycerol (0.2%) was added to this medium as an energy source.

An overnight culture was diluted and grown up to the logarithmic phase of growth. The cells were harvested by centrifugation, washed and resuspended in a fresh medium with a turbidity adjusted to 0.14 O.D. Glucose (0.5%) replaced glycerol unless otherwise indicated.

The synthesis of β-galactosidase was induced by incubating the cells in the presence of IPTG (2×10^{-4} M) at 37°C with aeration. For determination of total protein synthesis, ^{14}C-leucine (0.05 μc/ml) was added to the suspension. The activity of β-galactosidase and the incorporation of ^{14}C-leucine were assayed by methods similar to those described by Nakada and Magasanik (9), except that β-galactosidase activity was analysed at 37°C instead of 28°C. This activity was not affected by cAMP.

The induction of β-galactosidase formation was stopped either by addition of proflavin (60 μg/ml) or by removal of the inducer by filtering the induced suspension on a Millipore membrane (with pore size of 0.8 μ) and washing with cold medium. The filter was transferred into a flask containing cold medium and mixed vigorously. Aliquots from the heavy suspension obtained this way were diluted 1:5 in a prewarmed medium containing the appropriate additions. By this procedure, lasting about 3 min, the parallel suspensions contained the same number of cells.

Results and Discussion: Washed cells, resuspended in the growth medium, were incubated in the presence of IPTG and glucose. After 20 min, RNA formation was blocked by proflavin. The suspension was immediately divided into two portions, one of which received cAMP (3×10^{-3} M). The synthesis of β-galactosidase continued for about 10 min and then completely decayed. Fig. 1a shows that the amount of the enzyme formed in the presence of cAMP was higher than that formed in its absence, while under similar condi-

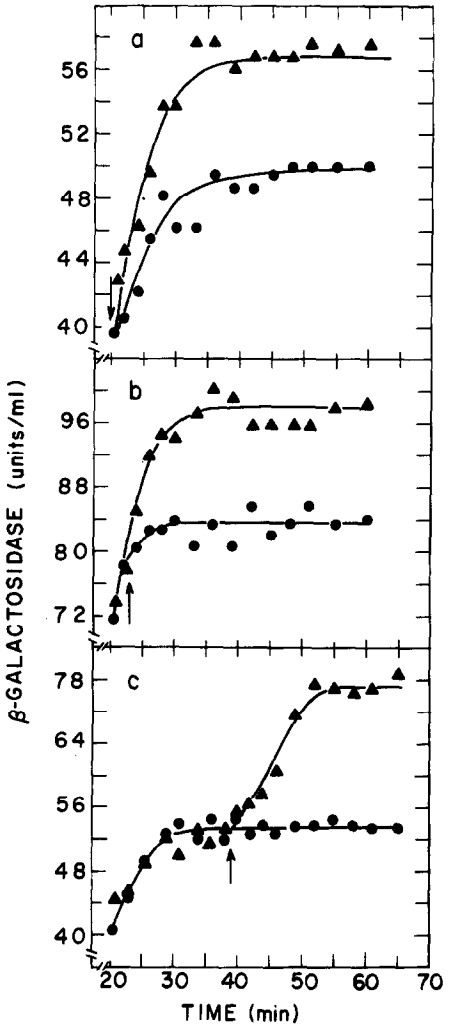

Figure 1. The effect of cAMP on β-galactosidase formation after addition of proflavin in cells preinduced in the presence of glucose.

Cells were harvested at the logarithmic phase of growth, washed and resuspended in the growth medium in a turbidity of 0.14 O.D. The suspension was incubated at 37°C with aeration, in the presence of 0.5% glucose and 2×10^{-4} M IPTG. After 20 min 60 μg/ml proflavin was added and the suspension divided into two portions. One of them (●) received nothing, while the other (▲) received 3×10^{-3} M cAMP at the following times, indicated by arrows: a) 20 min; b) 23 min; c) 39 min.

tions, cAMP had no effect on the incorporation of ^{14}C-leucine (Fig. 2). These findings suggest that cAMP facilitates the translation of the preformed lac-mRNA, but not that of the total mRNA. It could be argued that cAMP acted faster than proflavin, and that the

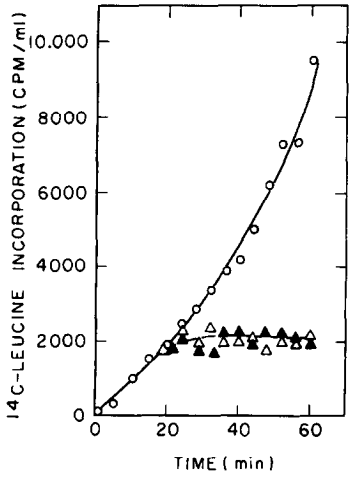

Figure 2. The effect of cAMP on ^{14}C-leucine incorporation after addition of proflavin.

A suspension prepared as described in the legend for Fig. 1 was incubated for 20 min with glucose and ^{14}C-leucine (0.05 μc/ml; 50 mC/mMole). The suspension was then divided into 3 portions, receiving respectively: (O) nothing; (Δ) 60 μg/ml proflavin; (▲) 60 μg/ml proflavin plus 3 x 10^{-3} M cAMP.

higher amount of the enzyme produced in its presence is due to an increase in the amount of lac-mRNA caused by cAMP before the manifestation of the effect of proflavin. This possibility was excluded by the following experiments: In the experiment described in Fig. 1b, cells were incubated with the inducer and glucose for 20 min. The induction was then terminated by proflavin, and cAMP was added to the appropriate portion of the suspension 3 min after proflavin. A similar stimulation of the residual synthesis of β-galactosidase by cAMP was obtained. In the experiment shown in Fig. 3, proflavin was added to cells at the same time as the addition of IPTG, glucose and ^{14}C-leucine in the presence or absence of cAMP. Both β-galactosidase synthesis and ^{14}C-leucine incorporation were completely arrested, and cAMP had no effect on either of them.

With these serving as controls for the efficiency of the effect of proflavin, the following experiment was performed. The cells were induced by IPTG for 20 min in the presence of glucose; proflavin was then added and cAMP added 19 min after proflavin - i.e.

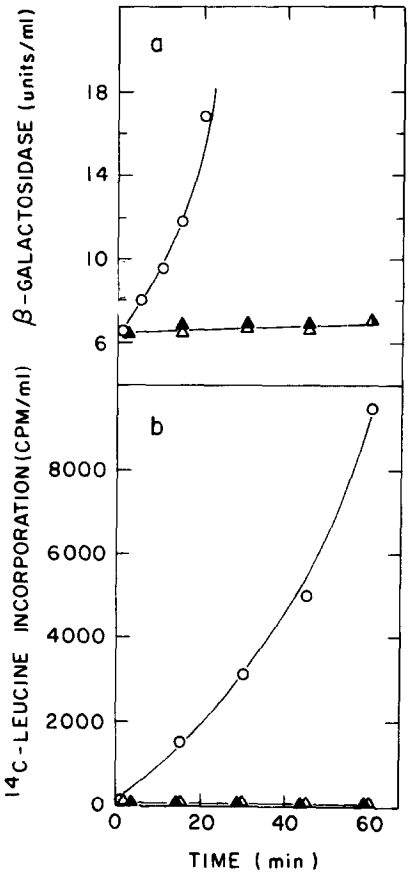

Figure 3. The efficiency of proflavin in blocking β-galactosidase induction and ^{14}C-leucine incorporation in the presence and absence of cAMP.

A suspension prepared as described in the legend for Fig. 1 was divided into 3 portions, receiving the following additions respectively: (O) IPTG + glucose + ^{14}C-leucine; (Δ) IPTG + glucose + ^{14}C-leucine + proflavin; (▲) IPTG + glucose + ^{14}C-leucine + proflavin + cAMP. Samples were dropped at different times for the assay of a) β-galactosidase formation, and b) ^{14}C-leucine incorporation.

after the residual synthesis of β-galactosidase had completely decayed. Even at this stage cAMP promoted new synthesis of β-galactosidase, which proceeded for a further period of about 10 min (Fig. 1c).

In other experiments, the induction of β-galactosidase formation was terminated by removal of the inducer after 20 min of incubation with IPTG and glucose. The washed

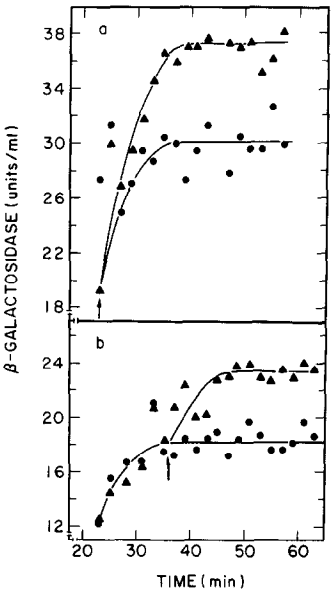

Figure 4. The effect of cAMP on β-galactosidase formation after removal of the inducer in cells preinduced in the presence of glucose.

Cells were induced to synthesize β-galactosidase in the presence of glucose for 20 min. The suspension was then filtered on a Millipore membrane (0.8 μ pore size) and washed with cold medium. The filter was transferred to a flask containing cold medium and mixed vigorously. Aliquots of 5 ml of the heavy suspension were transferred to two flasks containing 20 ml prewarmed medium, to which glucose was added. This procedure lasted about 3 min. One of the resulting suspensions (●) obtained nothing, and the other obtained cAMP at a) 23 min; b) 36 min, as indicated by arrows(▲).

cells were resuspended in a prewarmed medium containing glucose. Here, too, cAMP stimulated the residual synthesis of β-galactosidase, irrespective of whether it was added immediately after the removal of the inducer or when the enzyme formation had already decayed (Fig. 4). Similar results (in experiments not presented here) were obtained when the induction of β-galactosidase formation was stopped by 5-fluorouracil.

These results suggest that in the presence of glucose, only part of the lac-mRNA is translated, while the translation of the rest is repressed, and that cAMP overcomes this inhibitory effect by a reverse action on the translation of this part. Moreover, it appears that the untranslated lac-mRNA is stabilized - i.e. protected from the decay

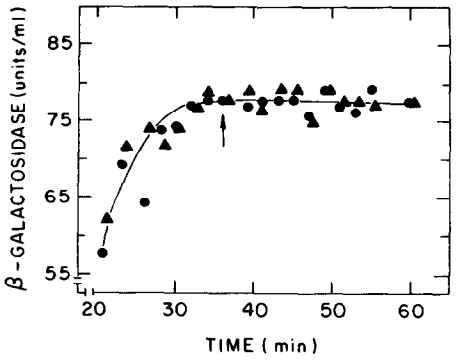

Figure 5. The effect of cAMP with glucose added 17 min after the addition of proflavin in cells preinduced in the absence of glucose.

Cells were induced in the absence of glucose. The suspension was divided after 20 min into two portions, each receiving proflavin. One received nothing (●), and the other (▲) received cAMP and glucose 17 min later, as indicated by arrow.

process, since its translation could be promoted by cAMP even if this compound was added after the enzyme synthesis had stopped.

In order to determine whether the stable lac-mRNA was specific for catabolite repression or whether it could exist also in its absence, cells were induced to synthesize β-galactosidase in the absence of a carbon source for 20 min, the induction was then stopped by proflavin, and the enzyme synthesis allowed to decay. The cAMP was then added together with glucose (the function of the latter is to allow the action of cAMP on intact cells (4)). Fig. 5 shows that under these conditions no stable lac-mRNA remained.

Another question was, whether the stable lac-mRNA had to be synthesized in the presence of glucose in order to become stable or whether even lac-mRNA synthesized in the absence of the sugar could be converted into the stable form after the addition of glucose. To clarify this, cells were incubated with IPTG in the absence of carbon source, and after 20 min the induction was stopped by proflavin and the suspension divided into 3 portions, all receiving glucose 2 min later. One portion, serving as

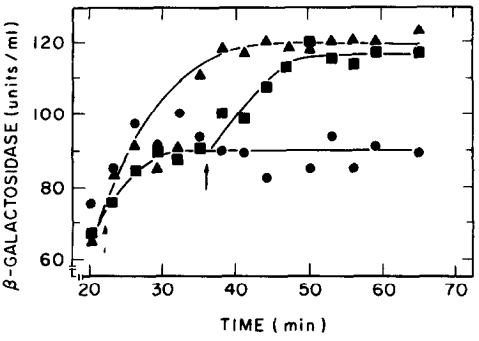

Figure 6. The effect of cAMP on β-galactosidase formation in the presence of glucose after the addition of proflavin in cells preinduced in the absence of glucose.

Cells were induced to synthesize β-galactosidase in the absence of glucose. At 20 min the suspension received proflavin and was divided into 3 portions, each receiving glucose 2 min later. One portion obtained cAMP at 22 min (▲), the second at 36 min (■), and the third did not obtain cAMP (●).

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a control, had no further additions, while another portion received cAMP at the same time as glucose, and the third received this compound after the enzyme synthesis had stopped. In both cases cAMP stimulated the enzyme formation (Fig. 6), suggesting that in the presence of glucose, lac-mRNA could be converted into the stable form even when this mRNA had been synthesized in the absence of sugar.

This view was further supported by the following experiment: Cells were induced to produce β-galactosidase and allowed to incorporate ^{14}C-leucine in the absence of carbon source. After 20 min, proflavin was added and the suspension was divided into two portions, with the addition of glucose to only one of them. Since no mRNA was newly produced in the presence of proflavin, any further protein synthesis taking place under these conditions could only be ascribed to the translation of the mRNA synthesized before the addition of the inhibitor. Glucose increased the ^{14}C-leucine incorporation; therefore the results were presented in Fig. 7 as a relative formation of β-galactosidase. These results show that the relative formation of the enzyme was lower in the presence of glucose than in its absence. This indicated that the translation of a part of the lac-mRNA was inhibited

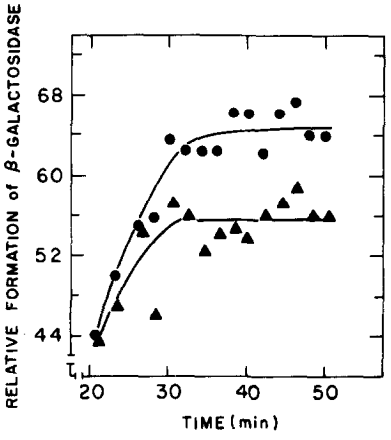

Figure 7. The effect of glucose on the relative formation of β-galactosidase after the addition of proflavin.

Cells were incubated with IPTG and ^{14}C-leucine in the absence of glucose. At 20 min the suspension was divided into two portions, one of which received proflavin (●) and the other proflavin and glucose (▲). The incorporation of ^{14}C-leucine was increased by glucose. In the experiment described here the incorporation obtained in the absence of glucose was 89% of that obtained in the presence of glucose. The results were accordingly corrected by multiplying the activity of β-galactosidase produced in the presence of glucose by 0.89 and presented as a relative formation of the enzyme.

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in the presence of glucose even if this mRNA had been produced in the absence of the sugar.

In summary, the results presented here indicate that the translation of a part of the lac-mRNA is repressed in the presence of glucose, irrespective of whether this mRNA was synthesized in the presence or absence of glucose. It has previously been reported (10,11, 12) that if translation is blocked by chloramphenicol, or amino acid starvation, or anaerobiosis, which keep ribosomes attached to the mRNA, this mRNA is protected from decay. It can similarly be proposed that in catabolite repression, the inhibition of the lac-mRNA translation is of a type which keeps the ribosomes attached to this mRNA and thus protects it from decay.

Several investigators have proposed that catabolite repression and cAMP affect the transcription of the lac-mRNA (4,5,6,9). According to the view that transcription and translation are coupled and in the light of our present results, it seems more likely that

any effect of cAMP and catabolite repression on the transcription, is a secondary result of the effect on the translation.

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