THE STEP SENSITIVE TO CATABOLITE REPRESSION AND ITS REVERSAL BY 3'-5' CYCLIC AMP DURING INDUCED SYNTHESIS OF β-GALACTOSIDASE IN E. COLI.

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It has been shown recently (Ullmann A. and Monod J., 1968 - Perlman R. and Pastan I., 1968) that 3'-5' cyclic AMP reverses catabolite repression (Magasanik B., 1961) of induced enzyme synthesis in E. Coli. The question whether the glucose effect and its antagonist 3'-5' cyclic AMP act upon transcription or upon translation has not been unambiguously answered.

This question has been reexamined using the early kinetics of induced β-galactosidase synthesis in E. coli 3000 (wild type) by means of pulse induction and a number of inhibitors acting on well defined steps of the process of gene expression. This method, (Kepes A., 1969), can distinguish the initiation and elongation processes of messenger RNA and of the polypeptide chain, and permits the measurment of the half life of the coding capacity of the preformed messenger.

To the inhibitors used previously (Kepes A., 1969, Leive L., 1965) another antibiotic, rifampicin which is known to inhibit initiation of new RNA chains (Sippel A. and Hartman G., 1968), has been added. The permeability of bacteria has been modified by EDTA treatment (Leive L., 1965 ;

Leive L., 1965) when actinomycin D or rifampicin were used.

Figure 1 represents an experiment in which an exponential culture of E. coli 3000 growing on medium 63 glycerol was submitted after EDTA treatment to a 90 seconds pulse induction by isopropyl β-D-thiogalactoside (IPTG) 0.1 mM. The induction was stopped by addition of 5 mM o-nitrophenyl-β-D-fucoside (ONPF), a competitive inhibitor of the inducer (Muller-Hill, B., Rickenberg H.V., and Wallenfels K., 1964).

The time course of expression of messenger RNA initiated during the pulse was followed by sampling on chloramphenicol 50 μg/ml at short intervals and measuring β -galactosidase activity on the chromogenic substrate o-nitrophenyl-β-D- galactoside (ONPG). It appears that addition of glucose or or of 3'-5' AMP after the pulse has no effect whatsoever on the extent or time course of subsequent enzyme synthesis, while addition of glucose together with the inducer drastically depresses the amount of β -galactosidase and addition of 3'-5' cyclic AMP with the inducer significantly increasses this amount. It can be concluded that the induction obtained without any supplement was not maximal due doubtlessly to a moderate catabolite repression in the EDTA treated cells on glycerol.

It is remarkable that the half life of the coding capacity of messenger RNA was not modified in any of the above conditions as illustrated by the semilog plots of the observed waves.

Similar results were found when the induction pulse was stopped by addition of rifampicin 1 ug/ml.

Glucose 6-phosphate, gluconate and a combination of gluconate plus glucose have been reported to cause catabolite

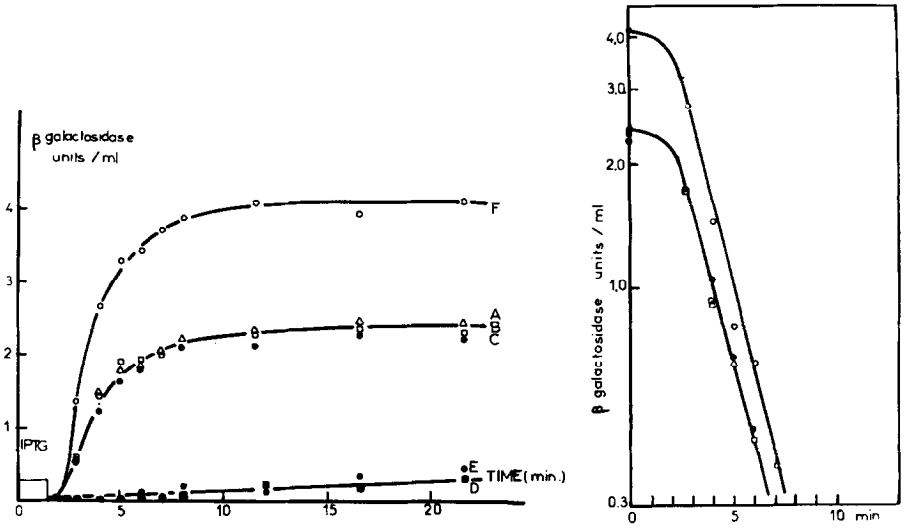

FIGURE 1 - EFFECT OF GLUCOSE AND CYCLIC AMP ON ELEMENTARY WAVES.

Strain E. coli 3000 growing exponentially in medium 63 glycerol B_1 was filtered, washed and resuspended in a buffer containing 120 mM Tris-Hcl pH 7.6. 1 mM EDTA was added and the suspension was incubated 1 min. at 37° with shaking and then diluted with 10 volumes of medium 63 glycerol. Final bacterial density 0.2 mg/ml. IPTG 0.1 mM was added at time 0 and, 5 mM ONPF 90 seconds later.

Bacteria were separated into six fractions :

	Addition during IPTG pulse :	Addition during deinduction
A	0	0
B	0	glucose 22 mM
C	0	cyclic AMP 5 mM
D	glucose 22 mM	-
E	glucose 22 mM	cyclic AMP 5 mM
F	glucose + cyclic AMP 5 mM	-

After deinduction samples were diluted in chloramphenicol 50 µg/ml.

At right, semi-logaritmic representation of the difference between β -galactosidase value at the plateau and at time t.

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repression in a mutant resistant to the glucose effect (Hsie A.W. and Rickenberg H.V., 1967). These substrates when present

during the pulse of induction of wild type E. coli were found fo decrease the yield of β-galactosidase by 70 to 90 percent. They had no effect when added after the pulse and their repressing effect during pulse induction is nearly completely reversed by the simultaneous addition of cyclic AMP 5 mM. This observation is at variance with the results of Goldenbaum and Dobrogrosz (1968).

Another way of establishing catabolite repression was to grow bacteria on a poor nitrogen source, e.g. glutamate instead of ammonia. Table I summarizes the effects of 3'-5' cyclic AMP under these conditions, when added either during or after the pulse induction respectively. It considerably increased the yield of β-galactosidase when added with the inducer but had no effect when added after it.

No effect of either glucose or of cyclic AMP on the translation steps and on the half life of the coding capacity of messenger RNA could be detected when the residual enzyme synthesis, after a short period of induction, was monitored

TABLE 1

THE EFFECT OF CYCLIC AMP DURING STRONG CATABOLITE REPRESSION.

mM cyclic AMP during induction pulse 2 minutes	mM cyclic AMP during expression 30 minutes	β-galactosidase units/ml
0	0	0.05
5	0.1	2.10
0	5	0.05

E. coli 3000 grown on medium with glutamate as nitrogen source and glucose as carbon source.

Bacteria are induced by IPTG 0.5 mM with or without cyclic 3'-5' AMP. Deinduction was done after 2 minutes by dilution 50 fold in the growth medium containing or not cyclic 3'-5'AMP.

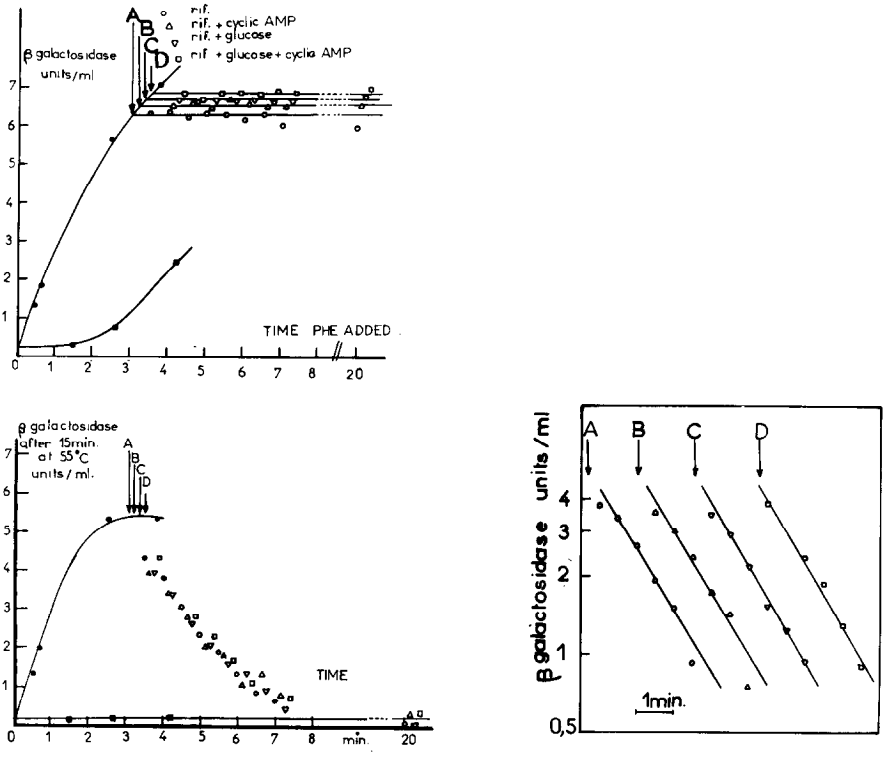

FIGURE 2 - COMPARISON OF THE EFFECT OF GLUCOSE AND CYCLIC AMP ON THE HALF LIFE OF PEPTIDE INITIATION CAPACITY OF PREFORMED MESSENGER RNA.

Strain E. coli 3000 grown on medium 63 glycerol B_1, with phenylalanine was submitted to the EDTA treatment described in legend of fig. 1 and supplemented with 0.5 mM p. fluorophenylalanine. After a few minutes, bacteria were induced by IPTG 0.1 mM at short intervals, samples were transferred into six volumes of a medium containing either rifampicin 1 µg/ml + L-phenylalanine 0.5 mM and incubated 20 minutes, either chloramphenicol 50 µg/ml.

3 minutes after induction the culture was separated into four fractions :

A - receiving rifampicin 1 µg/ml.
B - receiving rifampicin 1 µg/ml + cyclic AMP 5 mM.
C - receiving rifampicin 1 µg/ml + glucose 22 mM.
D - receiving rifampicin 1 µg/ml + cyclic AMP 5 mM + glucose 22 mM.

Samples of these deinduced cultures were transferred at short intervals on L-phenylalanine 0.5 mM and incubated 20 minutes.

Total β-galactosidase activity was mesured and plotted versus the time of addition of L-phenylalanine (upper figure).

Thermolabile β-galactosidase activity was inactivated by heatir 15 minutes at 55°C. The heat stable β-galactosidase is plotted versus the time of addition of L-phenylalanine.

At right, semi-logarithmic representation of the decreasing capacity of peptide initiation.

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by the incorporation of an aminoacid analog into β-galactosidase.

In the experiment depicted in figure 2, the growing culture was supplemented with parafluoro-phenylalanine before the addition of inducer. This is known to result in thermolabile β-galactosidase synthesis. Induction was stopped by addition of rifampicin alone or with glucose or with cyclic AMP or both, samples were transferred at different times on excess L-phenylalanine. The final yield of total and thermostable β-galactosidase was measured. The final yield of total enzyme activity was independent of the time of addition of phenylalanine while the yield of thermostable enzyme decreased. Since only the molecules synthesized entirely with phenylalanine exhibit full stability at 56°, the curves represent the decreasing capacity to initiate new peptide chains on the preformed messenger RNA. Irrespective of the presence of glucose or cyclic AMP or both, the total capacity to initiate new β-galactosidase chains was equal and decreased with a half life of 75 seconds.

This results strongly suggest that glucose and cyclic AMP act exclusively on transcription provided the assumption that their action is instantaneous according to the same

criteria as used for agents like rifampicin and actinomycin. If this assumption is born out it can be decided whether glucose and cyclic AMP act upon the same step as rifampicin (polynucleotide chain initiation) or as actinomycin (RNA chain elongation).

The experiment represented in figure 3 answers these questions. In this experiment, the transition from normal induced synthesis to catabolite repression was obtained by addition of glucose 40 seconds after the inducer and the reversal of catabolite repression by addition of cyclic AMP at 4 minutes. The amount of β- galactosidase messenger RNA initiated at any time was assessed after addition of rifampicin and the amount of messenger terminated after addition of actinomycin, by allowing the termination of all steps posterior to the inhibited one. It can be seen that on the rifampicin curve the effect of glucose and of cyclic AMP were instantaneous whereas on the actinomycin curve the effect was delayed by 80 to 100 seconds. This time is known to be necessary for the complete transcription of the Z segment of DNA at 37°, thus transcription time was not significantly modified by either glucose or cyclic AMP. In this experiment, glucose repressed the rate of messenger RNA initiation by about 90 % as compared to the bacteria growing on glycerol and cyclic AMP reversed this repression to reach a rate 1.15 times the control.

According to these results the catabolite repression by glucose and its reversal by cyclic AMP act on a step of the sequence of biosynthetic events, which is undistinguishable on the time scale from the step upon which rifampicin is acting and from the step influenced by inducer or competitive

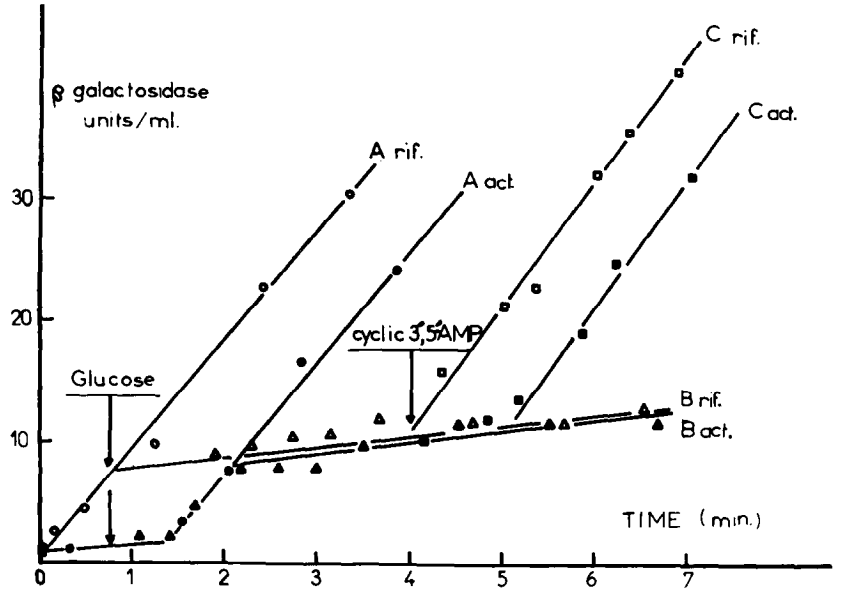

FIGURE 3 - EFFECTS OF GLUCOSE AND CYCLIC AMP ON TRANSCRIPTION.

A culture of E. coli 3000 in medium 63 glycerol B_1 was harvested in exponential growth and submitted to EDTA treatment. After a few minutes, cells were induced by IPTG 0.5 mM; this time is time 0 on the graph. At short intervals samples were diluted with 6 volumes of medium containing rifampicin 1 µg/ml (open symbols) or actinomycin D 10 µg/ml (full symbols), and incubated for a further 20 minutes.

At 90 seconds part of the induced culture (A) was supplemented with 22 mM glucose (B), at 4 minutes a part of the glucose repressed culture was supplemented with cyclic AMP 5 mM (C).

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inhibitors of the inducer. The resolution of the method can be safely estimated to be better than 10 seconds and probably about 5 seconds. This is to be compared with the 90 or 100 seconds necessary for the expression of the three gene products of the lactose operon. The chronological order in which the steps of induction, attachment and start of RNA polymerase occur in relation with the step affected by cata-

bolite repression is presently beyond experimental approach by the kinetic method.

In contrast, it can be concluded that catabolite repression and its reversal by cyclic AMP do not significantly modify the rate of RNA chain growth, the rate of peptide chain initiation, the yield of translation and the half life of the coding capacity of preformed messenger RNA, at least in short term experiments, and at least for the first gene product, β-galactosidase.

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