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Accumulation of Untranslated Lactose-Specific Messenger Ribonucleic Acid during Catabolite Repression in *Escherichia coli*

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When *Escherichia coli* K-12 Hfr.H was induced to synthesize β-galactosidase in the presence of glucose, an untranslated lactose-specific mRNA (lac-mRNA), protected from decay, was found to accumulate progressively within the cells. The lac-mRNA accumulation was unaffected by the carbon source on which the cells had been grown before the induction. The amount of the lac-mRNA available for translation was affected by catabolite repression and 3′:5′-cyclic AMP, but it remained unclear whether this was a direct effect on the formation of the lac-mRNA or a consequence of the effect on its translation.

It has been suggested that catabolite repression of β-galactosidase and some other enzymes results from depletion of the intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP), which apparently plays a role in the regulation of the synthesis of these enzymes (Makman & Sutherland, 1965; Perlman & Pastan, 1968*a*; De Crombrugghue, Perlman, Varmus & Pastan, 1969; Jacquet & Kepes, 1969; Chambers & Zubay, 1969).

Different views on the level at which catabolite repression and cyclic AMP exert their effects have been suggested. Magasanik and co-workers (Nakada & Magasanik, 1964; Silverstone, Magasanik, Reznikoff, Miller & Beckwith, 1969; Silverstone, Arditti & Magasanik, 1970), Kepes (1963), Attardi, Naono, Rouviere, Jacob & Gros (1963) and Sells (1965) have presented results suggesting that catabolite repression acts at the transcription level, whereas Yudkin & Moses (1969), Moses & Yudkin (1968) and Hauge, MacQuillan, Cline & Halvorson (1961) have shown that the translation is the step affected by catabolite repression. Other workers (Pastan & Perlman, 1968; Perlman & Pastan, 1968*a*,*b*; Perlman, De Crombrugghue & Pastan, 1969; De Crombrugghue, Varmus, Perlman & Pastan, 1970; Varmus, Perlman & Pastan, 1970; Jacquet & Kepes, 1969; Chambers & Zubay, 1969) concluded that cyclic AMP stimulated β-galactosidase synthesis in intact cells and cell-free systems by increasing the formation of lactose-specific mRNA (lac-mRNA), whereas Pastan & Perlman (1969) found that this nucleotide acted at the level of translation in the case of tryptophanase.

Aboud & Burger (1970) found that the translation of some of the lac-mRNA was blocked in the presence of glucose. We also observed that the untranslated lac-mRNA was protected from the decay process and that the translation of this mRNA was promoted by the addition of cyclic AMP to the medium.

The present study shows that the untranslated lac-mRNA progressively accumulates within the cells when the induction of β-galactosidase takes place in the presence of glucose. This study shows also that catabolite repression and cyclic AMP affect the amount of the lac-mRNA available for translation, but it provides no evidence that this is due to a direct action at the transcription level.

MATERIALS AND METHODS

Chemicals. [G-^{3}H]Uridine (6700mCi/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals used were as described by Aboud & Burger (1970).

Strain, medium and growth conditions. An overnight culture of *Escherichia coli* K-12 Hfr.H requiring thiamin, grown in the medium described by Aboud & Burger (1970) under static conditions at 37°C, was diluted in fresh medium and grown to the exponential phase with aeration at 37°C. Glycerol (0.2%, w/v) or glucose (1.0%, w/v) served as carbon source according to the experimental requirements. The cells were harvested by centrifugation, washed and resuspended in the growth medium. The turbidity of the culture, measured with a Klett–Summerson photoelectric colorimeter, was adjusted to an extinction of 0.12 E_{540} unit.

Induction and assay of β-galactosidase. These were as described by Aboud & Burger (1970).

Incorporation of [1-^{14}C]*leucine and* [^{3}H]*uridine.* Total protein synthesis was determined by the incorporation of [1-^{14}C]leucine (0.05μCi/ml added together with 50μg/ml of non-radioactive leucine) into hot-5%(w/v)-trichloroacetic acid-insoluble material. RNA synthesis was determined by the incorporation of [^{3}H]uridine (10μCi/ml) into cold-5%-trichloroacetic acid-insoluble material. In both

cases the insoluble material was collected on Millipore membrane filters (pore size 0.8 μm), washed with cold 5% trichloroacetic acid and the radioactivity was counted in scintillation liquid composed of 200 mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (dimethyl POPOP) and 4 g of 2,5-diphenyloxazole (PPO) in 1 litre of toluene.

RESULTS

Inhibition of RNA synthesis by proflavine. Proflavine was shown, in control experiments described by Aboud & Burger (1970), to be an efficient inhibitor of RNA synthesis. When this compound was added together with the inducer of β-galactosidase, at a time in which no lac-mRNA existed in the cells, no enzyme formation could be detected. However, if it was added a certain time after the inducer, when lac-mRNA already existed in the cells, a residual synthesis of β-galactosidase continued until all the preformed lac-mRNA was degraded. Fig. 1 provides more direct evidence

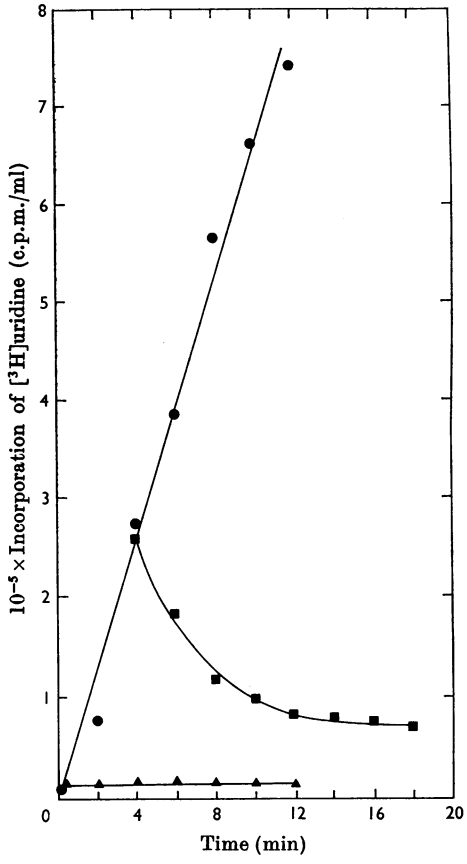

Fig. 1. Inhibition of mRNA formation by proflavine. Washed cells of *E. coli* were resuspended in a medium containing glucose (1.0%, w/v). The culture received [^{3}H]uridine (10 μCi/ml) and was immediately divided into three portions. Proflavine (50 μg/ml) was added at zero time to one of the portions (▲) and 4 min later to another (■), whereas the third, serving as a control, did not receive proflavine (●).

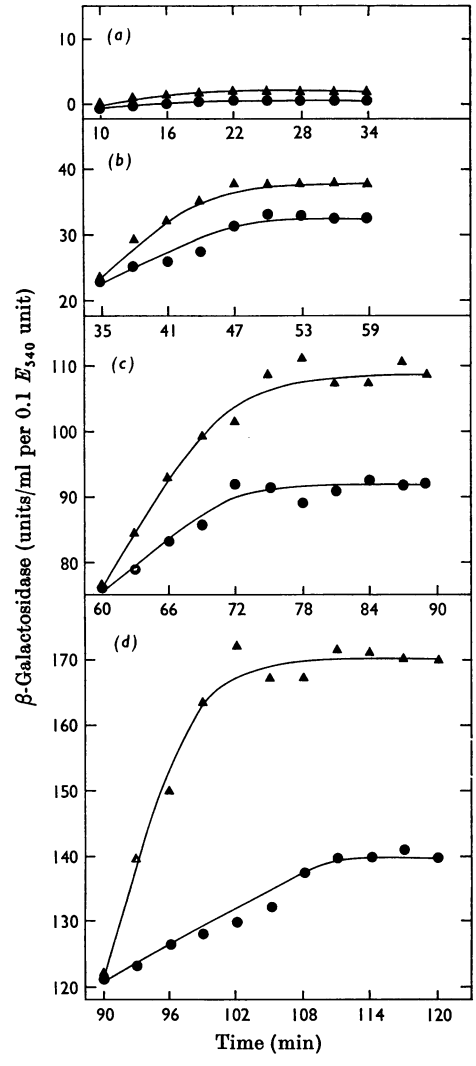

Fig. 2. Accumulation of lac-mRNA in glycerol-pregrown cells. Cells pregrown to the exponential phase in a medium containing glycerol (0.2%, w/v) were washed and resuspended in fresh growth medium to a turbidity equivalent to 0.12 E_{540} unit. At zero time isopropyl thio-β-D-galactoside (0.2 mM) and glucose (1.0%, w/v) were added and the culture was agitated at 37°C. Two equal portions were taken from the culture at (*a*) 10 min, (*b*) 35 min, (*c*) 60 min and (*d*) 90 min. Both portions received proflavine (50 μg/ml) and were allowed to produce β-galactosidase in the presence of cyclic AMP (3.0 mM) (▲) and in its absence (●). The turbidity of the portions was measured and the enzyme activity was expressed as units/ml per 0.1 E_{540} unit.

for the efficiency of proflavine in blocking RNA synthesis. *E. coli* culture was divided into three portions, all receiving [^{3}H]uridine at time zero. Proflavine was added immediately to one of the portions and 4min later to the second, whereas the third, serving as a control, was incubated without addition of proflavine. No significant incorporation of [^{3}H]uridine occurred when proflavine was added at zero time, and an immediate decline in the radioactivity of the cold-5%-trichloroacetic acid-insoluble material was detected when it was added after 4min. This decline was due to the breakdown of the labelled preformed mRNA (see also Artman & Engelberg, 1965; Nierlich, 1967).

Accumulation of lac-mRNA in glycerol-pregrown cells. Washed cells of *E. coli*, pregrown in glycerol-containing medium, were induced to synthesize β-galactosidase in glucose-containing medium. The growth of the culture was followed by turbidimetric measurements. Proflavine was added to two equal portions taken from the culture at different times as indicated in Fig. 2. The enzyme formation was allowed to occur in the presence of cyclic AMP in one portion and in its absence in the other, and was determined for a bacterial density equivalent to an extinction value of 0.1 E_{540} unit in each case. The difference between the amounts of the enzyme formed in the presence and in the absence of cyclic AMP after the blocking of further RNA synthesis represented the lac-mRNA, the translation of which was blocked by glucose and promoted by cyclic AMP. The results presented in Fig. 2 show that this mRNA progressively accumulated within the cells. This accumulation proceeded for at least 90min.

Accumulation of lac-mRNA in glucose-pregrown cells. Yudkin (1969), studying the role of the carbon source on which cells were pregrown in the response of β-galactosidase synthesis to glucose, concluded that glycerol and glucose produced selective conditions for the growth of different metabolic mutants, in which β-galactosidase synthesis differed in its response to glucose. This and the contradictory reports on the step affected by catabolite repression and cyclic AMP raised the possibility that different carbon sources might select mutants that varied in the site sensitive to catabolite repression and to cyclic AMP. To clarify this, cells pregrown in glucose-containing medium were tested, by the same method, for the accumulation of untranslated lac-mRNA. Fig. 3 shows (*a*) that cyclic AMP acts at the translation level in such cells too and (*b*) that these cells accumulate untranslated lac-mRNA as well as glycerol-pregrown cells. Moreover, similar results were obtained with cells pregrown in 12 subsequent subcultures in glucose-containing medium (Fig. 4). These results indicate that the carbon source on which the cells were pregrown had no effect on the site sensitive to catabolite repression and cyclic AMP or on the ability of the cells to accumulate the untranslated lac-mRNA.

Lac-mRNA content in the presence and in the absence of cyclic AMP. Another problem to be

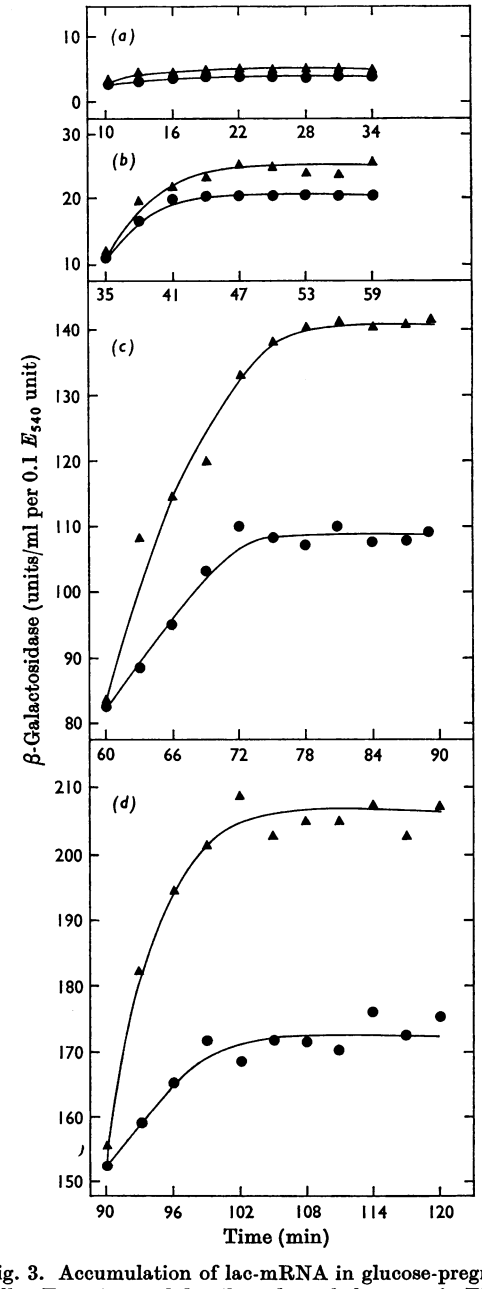

Fig. 3. Accumulation of lac-mRNA in glucose-pregrown cells. Experimental details and symbols are as in Fig. 2, except that the cells were pregrown to the exponential phase in glucose-containing medium.

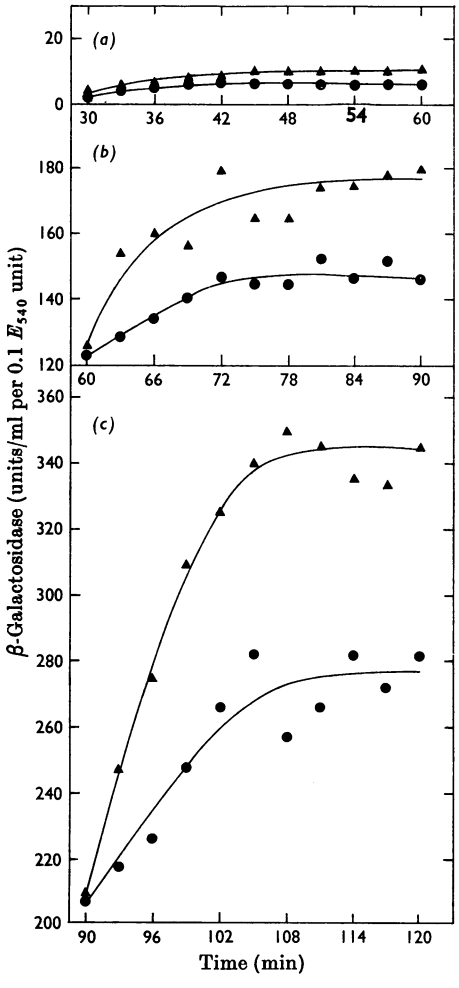

Fig. 4. Accumulation of lac-mRNA in cells pregrown in 12 subcultures in glucose medium. Cells grown to the exponential phase in glucose-containing medium were diluted 1:100 in a fresh glucose-containing medium. This was repeated 12 times and then the cells were tested for lac-mRNA accumulation, as described in Fig. 2, in portions taken at (*a*) 30 min, (*b*) 60 min and (*c*) 90 min.

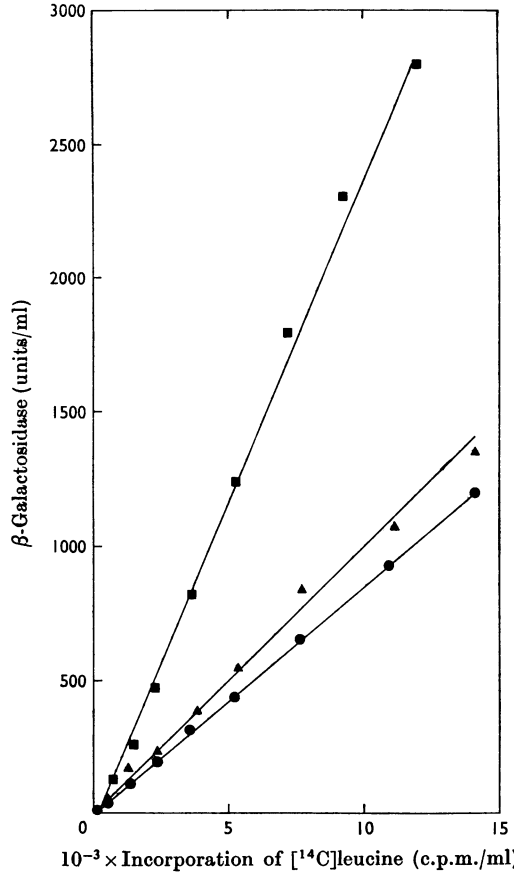

Fig. 5. Effect of cyclic AMP on the lac-mRNA content. Two portions of a culture of glycerol-pregrown cells were induced to form β-galactosidase in a medium containing glucose (1.0%, w/v), [^{14}C]leucine (0.05 μCi/ml) and non-radioactive leucine (50 μg/ml), one in the presence of cyclic AMP (3.0 mM) and the other in its absence. From the portion induced in the absence of the cyclic AMP two parallel samples were taken at different intervals and were incubated for a further 30 min with proflavine (●) and with proflavine + cyclic AMP (▲). Samples were also taken from the portion induced in the presence of cyclic AMP and incubated for a further 30 min with proflavine (■). At the end of the 30 min the samples were tested for enzyme and [^{14}C]leucine content.

clarified was whether the untranslated lac-mRNA, accumulated within the cells, together with that portion of the lac-mRNA that was translated in spite of the catabolite repression, represented the overall amount of the lac-mRNA that would be available for translation into active enzyme, under the same nutritional conditions, if the effect of catabolite repression was abolished. For this purpose glycerol-pregrown cells were induced to form β-galactosidase in a medium containing glucose and [^{14}C]leucine. The culture was immediately divided into two portions, one of which received cyclic AMP. At different intervals two parallel samples were taken from the portion without cyclic AMP and incubated, one with proflavine and the other with proflavine and cyclic AMP, for a further 30 min, to allow the lac-mRNA preformed in the absence of cyclic AMP to be translated in the presence and in the absence of the nucleotide. After 30 min the samples were tested for enzyme content and [^{14}C]leucine incorporation, and the enzyme

activity was plotted against radioactivity to describe the differential rates of the formation of the overall lac-mRNA (as detected in the samples incubated with proflavine and cyclic AMP) and of the lac-mRNA that was translated during catabolite repression. The difference between them represented the differential rate of the accumulation of the untranslated lac-mRNA. Samples from the portion induced to form β-galactosidase in the presence of cyclic AMP were transferred into flasks containing proflavine and then were also incubated for a further 30 min. If the amount of the lac-mRNA available for translation was unaffected by catabolite repression and cyclic AMP, the differential rate of the enzyme synthesis in the culture, induced in the presence of cyclic AMP, should be the same as that in the samples taken from the culture induced in the absence of the nucleotide but incubated with proflavine and cyclic AMP. Fig. 5 shows that this was not the case. The differential rate observed in the culture induced in the presence of cyclic AMP was twice that observed in the cells induced in the absence of the nucleotide. The differential rate of the accumulation of the untranslated lac-mRNA was about 10% of the increase of the differential rate of the enzyme formation caused by cyclic AMP.

DISCUSSION

Different views as to whether catabolite repression and cyclic AMP exert their effect at the level of mRNA transcription or at that of its translation have been proposed. Aboud & Burger (1970) showed that glucose prevents translation of some of the lac-mRNA and that the untranslated lac-mRNA is protected from decay. Similar protection of mRNA has been observed by others (Fan, Higa & Levinthal, 1964; Nakada & Fan, 1964; Lindahl & Forchhammer, 1969) when translation was interfered with by chloramphenicol, amino acid starvation or anaerobiosis, but not by puromycin. This suggests that the inhibition of the lac-mRNA translation by catabolite repression is of a type that protects the mRNA from degradation, possibly by keeping the ribosomes attached to it. We have also shown (Aboud & Burger, 1970) that the translation of the protected lac-mRNA was promoted by cyclic AMP.

The present study provides further support for this view. It shows that the untranslated lac-mRNA accumulates within the cells for a considerable time (Figs. 2 and 5). Such accumulation suggests that during this time the untranslated lac-mRNA is protected from decay. Protection and accumulation of untranslated lac-mRNA were observed in cells pregrown in glucose-containing medium (Figs 3 and 4) as well as in glycerol-containing medium (Fig. 2), indicating that the carbon source on which the cells were pregrown had no effect in this respect.

The results presented in Fig. 5 show that the amount of the lac-mRNA available for translation produced in the presence of cyclic AMP was about twice that produced in its absence. These results do not exclude the possibility that transcription of the lac-mRNA is also affected by catabolite repression and cyclic AMP. However, they do not prove unequivocally that the transcription was affected directly. In accordance with the view that transcription and translation are linked (Byrne, Levin, Bladen & Nirenberg, 1964; Alpers & Tomkins, 1966; Shih, Eisenstadt & Lengyel, 1966; Kepes, 1967; Leive & Kollin, 1967; Miller, Hamkalo & Thomas, 1970) one can postulate that inhibition of the lac-mRNA translation by catabolite repression consequently halts its formation, and similarly that stimulation of the translation by cyclic AMP is accompanied by an increase of the transcription. It is also possible that transcription is not affected at all by catabolite repression and cyclic AMP, and that only the translation is affected. The fact that catabolite repression decreases the amount of the lac-mRNA available for translation, and that cyclic AMP does the reverse, can be speculatively explained by assuming that during catabolite repression only a small portion of the untranslated lac-mRNA accumulates, the rest being broken down and no longer available for translation. Cyclic AMP could prevent this by increasing the efficiency of translation, so that more lac-mRNA is translated into active enzyme, though the rate of transcription remains the same.

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