SYNTHESIS OF β-GALACTOSIDASE MESSENGER RNA DURING AMINO ACID STARVATION OF STRINGENT AND RELAXED STRAINS OF ESCHERICHIA COLI

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Received May 20, 1974

SUMMARY

The synthesis of β-galactosidase mRNA is apparently initiated but not completed during starvation of stringent strains for a required amino acid. The mRNA can be completed in the absence of inducer, during subsequent addition of the amino acid. In contrast to this finding, isogenic relaxed strains cannot initiate lac mRNA synthesis during amino acid starvation and/or complete it during subsequent growth. Thus, the transcription of lac mRNA during inhibition of protein synthesis by amino acid starvation is affected by the rel genotype of the bacterial strains used. Since under these conditions of amino acid starvation global mRNA production in the relaxed strain is not blocked, it is suggested that the control of lac mRNA is distinct from that which regulates global mRNA synthesis.

INTRODUCTION

There is a good deal of confusion concerning mRNA synthesis in relaxed and stringent strains of Escherichia coli. Lavallé and De Hauwer (1) reported that tryptophan mRNA was made equally in tryptophan starved cells of relaxed and stringent strains. On the other hand, Edlin, et al. (2) presented data which showed that, under similar conditions of amino acid starvation, the tryptophan mRNA concentration was ten times as great in the stringent strain as in the relaxed strain. In contrast to both of the above findings, Stubbs and Hall (3) found that during starvation for arginine the level of tryptophan mRNA decreased markedly in both stringent and relaxed strains. It has been found (1,3-5) that during amino acid starvation, the synthesis of other species of E. coli mRNA was only slightly inhibited.

The work on the synthesis of lac mRNA in the past was hampered by the difficulty in overcoming catabolite repression during amino acid starvation

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and during subsequent recovery from this starvation. For example, in order to overcome catabolite repression, much of the work on β-galactosidase mRNA synthesis during amino acid starvation was done under conditions which imposed a simultaneous starvation for glucose (6). With the availability of cyclic AMP as an agent to overcome catabolite repression (7), the previous work could be reevaluated.

The object of the present study was to determine whether functional lac mRNA is made during inhibition of protein synthesis by amino acid starvation and its dependence on the rel genotype of the strains used. Kennell and Simmons (8) have already reported that only approximately 35% of the β-galactosidase messenger is transcribed during leucine starvation. No full-length molecules are made. These incomplete molecules of β-galactosidase mRNA can be completed during the subsequent growth in the absence of inducer. We further report that the initiation and/or subsequent completion of lac mRNA is affected by the rel genotype of the bacterial strain used.

MATERIALS AND METHODS

Bacterial strains, growth medium and culture conditions. E. coli 15t rel^+ and 15t rel^- which require thymine, arginine and uracil and H128, rel^+ derived from CP78 and H130, rel^- derived from CP79, which require threonine, leucine, histidine, arginine, methionine and thiamine were used. Both pairs of strains are isogenic except for the rel locus. CP78 and CP79 were supplied by Dr. J.D. Friesen and 15t rel^+ and rel^- were supplied by Dr. P.S. Cohen. The cells were grown on Medium A (9) supplemented with the above requirements and 0.5% glucose. All cultures were grown at 37°C with vigorous aeration. All cultures were exponentially growing when used and were at a concentration of approximately 3×10^8 cells/ml when used or manipulated for the indicated experiments.

Procedures for amino acid starvation of cultures. Growing cells were rapidly washed by filtration on membrane filters (Millipore Corp.; 47 mm diameter, 0.65 micron pore size). The bacteria collected on the filter were washed several times with room temperature basal salts medium (Medium A, lacking the other nutrients). The bacteria were then suspended in the appropriate medium warmed to 37°C and lacking amino acid.

β-Galactosidase assay. The enzyme was determined as previously

described (10). The cells were induced with 0.5 mM isopropyl-β-D-thio-galactoside (IPTG) in the presence of 5 mM cyclic AMP. Enzyme activity is expressed in units per 10^9 cells. One unit of enzyme produces 1 nmole of o-nitrophenol per min at 37°C.

Preparation of labeled RNA. The procedure for isolation of labeled RNA has been previously described (11).

Determination of lac mRNA content of cells. The fraction of lac mRNA in the pulse-labeled RNA was determined by hybridization with λlac DNA immobolized on nitrocellulose filters, as previously described (11). All values were corrected for RNA interactions with λ DNA, by performing simultaneous hybridizations with λ DNA.

RESULTS

Loss of capacity of relaxed cells to synthesize β-galactosidase during amino acid starvation.

In all the experiments to be described in this investigation induction was carried out using isopropyl-β-D-thio-galactoside (IPTG, 0.5 mM) and cyclic AMP (5 mM) to minimize the effect of catabolite repression on RNA synthesis. Some of these experiments were also performed in the presence of up to 50 mM cyclic AMP with the same results obtained. This large concentration of cyclic AMP is necessary because lower concentrations do not overcome catabolite repression under all conditions. All experiments were done using the isogenic stringent and relaxed pairs, 15t *rel*$^+$ and 15t *rel*$^-$ and H128 and H130. The results obtained with each pair were the same and some data using 15t *rel*$^+$ and 15t *rel*$^-$ and some data using H128 and H130 are presented. (All experiments were also performed using glycerol as carbon source and the same results were obtained).

The synthesis of β-galactosidase mRNA during amino acid starvation was determined by the method of Kepes (12), which involved measuring the additional amounts of β-galactosidase formed after enzyme repression is re-established by washing out inducer. In this type of experiment cells are induced during amino acid starvation and then allowed to synthesize β-galactosidase in the absence of inducer, after addition of the required amino acid. Thus enzyme induction is separated from enzyme production and the latter is assumed to be a reflection of the amount of β-galactosidase mRNA synthesized during amino acid starvation.

The capacity of the relaxed strain H130 to be induced to make mRNA during methionine starvation decreased with increased duration of starvation. As can be seen in Figure 1, after 35 min starvation the relaxed strain possessed

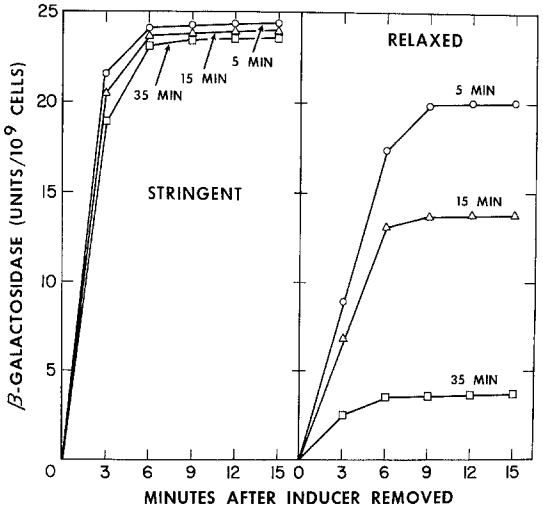

FIGURE 1: Capacity of relaxed and stringent strains to synthesize β-galactosidase mRNA during amino acid starvation. Cells of strains H128 and H130 were harvested and washed free of methionine by rapid filtration and washing. Each strain was suspended in Medium A (9) containing cyclic AMP and IPTG but lacking methionine. At each of the indicated times, 10 ml of each culture were rapidly washed by filtration and suspended in complete growth medium containing methionine but lacking cyclic AMP and IPTG. The amount of β-galactosidase synthesized at the indicated intervals was determined, as previously described (10). A similar experiment in which the starved cells were induced 3 min before harvesting (rather than continuously during starvation as above) gave the same results. All cultures were grown at 37°C with vigorous aeration and were exponentially growing at approximately 4 x 10^8 cells/ml when used or manipulated for the indicated experiments.

only about 20% of its original capacity to synthesize β-galactosidase. In contrast to this finding, the capacity of the stringent strain H128 to make β-galactosidase was unchanged after 35 min methionine starvation.

It is possible that mRNA is made during amino acid starvation of the relaxed strain but that it is not translated during subsequent growth. Consequently, no β-galactosidase would be made. This is unlikely, because direct determination of the lac mRNA content of the cells, by specific DNA-RNA hybridization also shows that little lac mRNA is present (at 35 min

starvation). Under these conditions only 0.05% of the RNA made during induction in the absence of methionine at 35 min was lac specific. This should be compared to the values obtained in growing induced (0.52% lac specific) and uninduced cells (0.02% lac specific). Consequently, in confirmation of the data presented in Figure 1, little lac mRNA is made during 35 min amino acid starvation of the relaxed strain. Protein synthesis, as determined by incorporation of radioactive amino acid, is approximately the same in recovering stringent and relaxed cells. Since it is known that total mRNA is synthesized in relaxed cells during amino acid starvation (data not shown and 1, 3-5) the results indicate that amino acid starvation of the relaxed strain exerts a selective effect on β-galactosidase mRNA synthesis.

We have also shown that lac mRNA is initiated during induction of starved stringent cells, but finished only during subsequent growth (in the absence of inducer). This was shown by the following experiment in which the synthesis of β-galactosidase was determined in the presence of rifampicin or actinomycin D during growth subsequent to induction. Rifampicin inhibits initiation but not elongation of RNA chains, whereas actinomycin D inhibits elongation. Consequently, we could determine, by compairing the level of β-galactosidase made in the presence of these drugs, whether new initiations and/or elongation (or both) of lac mRNA chains occurred during the subsequent growth in the absence of inducer. The results in Table 1 show that enzyme is made in cells treated with rifampicin but not in cells treated with actinomycin D. We are, therefore, left with the conclusion that lac mRNA synthesis is initiated during amino acid starvation of the stringent but not completed until subsequent growth in the absence of inducer.

Induction of β-galactosidase synthesis during recovery from amino acid starvation.

It had previously been reported that relaxed cells are impaired in their ability to synthesize inducible enzymes during recovery from extended periods of amino acid starvation, whereas constitutive enzyme synthesis was normal during the same period. Stringent cells were unimpaired in the synthesis of either inducible or constitutive enzymes (13). We have also verified this (data not shown). The mechanism by which this selective effect in inducible enzymes is achieved is not known.

Surprisingly, however, it was observed (Fig. 2) that cells induced

TABLE 1

Effect of inhibitors of RNA synthesis on
β-galactosidase synthesis during recovery
of the stringent strain from amino acid starvation

Additions to growth medium during recovery	β-galactosidase synthesized (units/10^9 cells)
a. Water (control)	22
b. Rifampicin	34
c. Actinomycin D	0

Growing cells of strain 15t $\underline{rel}^+$ were treated with ethylene-diamine tetraacetate as previously described (8), and then suspended in a growth medium lacking arginine. The cells were induced by the addition of IPTG and cyclic AMP, and the cultures were incubated with shaking for 7 min. Then the cells were rapidly washed free of IPTG and cyclic AMP by filtration. The cells were resuspended in Medium A (9) (containing arginine but no cyclic AMP or IPTG) and divided into 3 separate flasks containing, (a) water (control), (b) rifampicin (50 μg/ml) and (c) actinomycin D (50 μg/ml). The flasks were incubated at 37°C for 20 min with shaking and the amount of enzyme made was determined.

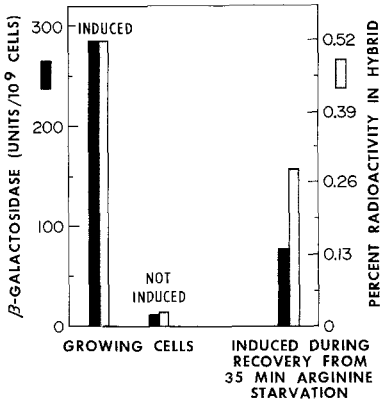

FIGURE 2: Lac mRNA content of growing cells, and of cells recovering from amino acid starvation. The experiments were performed as indicated in the legend to Figure 1. Only the 15t relaxed strain was used. RNA was isolated from cells pulse labeled with [^{3}H] adenine (12 μCi/ml; 3.3 Ci/mmole), for 2 min during growth (induced and uninduced), and from cells labeled for 3-5 min, induced during recovery from starvation for arginine. RNA was isolated from 25 ml cultures and was hybridized to λ lac DNA as previously described (11). The enzyme levels were obtained from the average plateau values obtained in several experiments similar to those described in Figure 1 and in a previous study (11).

during recovery from arginine starvation contain an inordinately high amount of lac mRNA (0.24% lac specific) compared to the amount of β-galactosidase synthesized (77 units). As can be seen, in these cells the lac mRNA content is about twice that amount which would be expected to be necessary for the synthesis of the amount of enzyme formed. Growing cells induced in the presence of cyclic AMP produced about 285 units of enzyme/10^9 cells and 0.52% of pulse labeled RNA was lac specific.

DISCUSSION

The synthesis of lac mRNA is apparently initiated but not completed during starvation of a stringent strain for a required amino acid. The mRNA initiated in the absence of the required amino acid can be completed during subsequent growth in the presence of the required amino acid, but in the absence of the added inducer and cyclic AMP. These results are in agreement with those presented previously by Kennell and Simmons (8). However, we find that the synthesis of β-galactosidase mRNA is severely inhibited during amino acid starvation of the isogenic relaxed strain compared to the stringent strain. These results together with parallel measurements of global mRNA in similar bacterial cultures (1,3-5) which showed that under these conditions of starvation the inhibited cells synthesized global mRNA in about the same amount as growing cultures, suggest that lac mRNA synthesis in relaxed cells is regulated by a control mechanism distinct from that which regulates total mRNA synthesis. Perhaps this regulation is at the level of synthesis and stability of ppGpp or other components specific for lac mRNA synthesis or stability. Inhibition of protein synthesis by antibiotics or by K^+- depletion also markedly interferes with lac mRNA accumulation (11,14,15). A trivial explanation of these results is that cyclic AMP cannot get into the starved relaxed cells, and therefore catabolite repression is not relieved. Since we have used up to 50 mM cyclic AMP and have obtained the same results using cells made permeable by treatment with ethylene-diamine tetraacetate (data not shown), we feel that catabolite repression plays no role in the observed phenomenon.

Another observation made during these studies is that there is no obligatory connection between transcription of the lac operon and its translation. Lac mRNA synthesized by relaxed strains (and not by isogenic stringent strains) immediately upon restoration of the required amino acids to starved

cells may not be fully translated into functional enzyme. About twice as much lac mRNA is apparently made than is translated. This conclusion which is based on the premise that the lac specific RNA made during recovery is potentially functional, is currently being investigated further.

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