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Dissociation of Lac Messenger Ribonucleic Acid Transcription from Translation During Recovery from Inhibition of Protein Synthesis

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Studies were made of the synthesis of Lac messenger ribonucleic acid (mRNA) by *Escherichia coli* in the absence of protein synthesis and of the coupling of transcription of *lac* operon to translation. Lac mRNA was not synthesized in the presence of chloramphenicol, and its synthesis steadily decreased during K^+ deprivation and treatment with puromycin. Since under these conditions total mRNA synthesis is not inhibited it is suggested that the control of Lac mRNA is distinct from that which regulates total mRNA synthesis. Lac mRNA synthesized during recovery from K^+ starvation or from chloramphenicol inhibition is not translated into functional enzyme, suggesting translational control over β-galactosidase synthesis.

The present study is concerned (i) with the synthesis of Lac messenger ribonucleic acid (mRNA) in the absence of protein synthesis and during recovery from unbalanced growth due to selective inhibition of protein formation; and (ii) with the coupling of *lac* operon transcription to translation. The availability of reliable methods for Lac mRNA determination (21) and β-galactosidase assay (17) and the feasibility of separating enzyme induction from enzyme production (12) make the β-galactosidase system a unique model for studies on the interrelationship between protein synthesis and the synthesis of stable RNA, total mRNA, and specific Lac mRNA. This work in the past was hampered by the knowledge that catabolite repression of β-galactosidase was severe in nonproliferating or slowly growing cultures, especially those incubated in glucose-containing media (14). With the discovery that cyclic adenosine monophosphate (AMP) relieves glucose repression, this obstacle has been removed (19).

We have studied the synthesis of Lac mRNA in *Escherichia coli* during inhibition of protein synthesis and during the initial period of recovery from such inhibition. The arrest of protein synthesis was achieved by treating cells with chloramphenicol or puromycin, by methionine starvation of a Rel^+ amino acid auxotroph, or by K^+ deprivation of a mutant unable to accumulate this ion from the growth medium. In a previous study we examined Rel^- cells starved of methionine (Artman and Ennis, *in manuscript*).

To establish whether coupling of transcription to translation is obligatory, we determined whether the Lac mRNA synthesized under the different culture conditions was always translated into functional enzyme. In this investigation we show that the ability of cells to synthesize β-galactosidase mRNA during inhibition of protein synthesis brought about by chloramphenicol or puromycin treatment or by K^+ deprivation is severely impaired. Furthermore, our study shows that Lac mRNA is not, under all conditions, translated into functional enzyme, suggesting a translational control over β-galactosidase synthesis.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. *E. coli* B207 (*his*$^-$, *leu*$^-$, *met*$^-$, *rel*$^+$, Sm^R), a mutant of *E. coli* B that is defective in its ability to concentrate and accumulate K^+ from the growth medium, was used (5). The cells were grown in Medium A (3) supplemented with glucose (0.25%) and histidine, leucine, and methionine (100 μg/ml each). In media lacking K^+, the K^+ phosphates were replaced by an equimolar concentration of Na^+ phosphates. All cultures were grown at 37 C with vigorous aeration and were exponentially growing at approximately 3×10^8 cells/ml when used or manip-

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ulated for the indicated experiments.

Inhibition of protein synthesis. Growing cells were rapidly washed by filtration on membrane filters (Millipore Corp.; 47-mm diameter, 0.65-μm 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. (i) Chloramphenicol (100 μg/ml) was present in the complete growth medium. (ii) Puromycin (300 μg/ml) was present in the complete growth medium. (iii) K^+ depletion: the cells were washed with basal salts medium containing Na^+ phosphates substituted mole for mole for the K^+ phosphates. After being washed, the cells were suspended in complete growth medium containing Na^+ in place of K^+. Adenosine, guanosine, and uracil (20 μg/ml each) were added because optimal RNA synthesis under these conditions requires the presence of these compounds (6). (iv) Methionine starvation: the cells were suspended in complete medium lacking methionine.

β-Galactosidase assay. The enzyme was determined as previously described (17). The cells were induced with 10^{-3} M isopropyl-β-D-thio-galactoside (IPTG) in the presence of 10^{-3} M 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 labeling RNA has been previously described (8).

Purification of phage and preparation of λ Lac DNA. λ $CI_{857}S_7$plac 5 i^-y^- was isolated from *E. coli* G141 (kindly supplied by D. Kennell) by temperature induction as previously described (21). The phage was purified and the deoxyribonucleic acid (DNA) was isolated by methods which were previously described (10, 24).

Determination of Lac mRNA content of cells. The fraction of Lac mRNA in the pulse-labeled RNA was determined by hybridization with λ Lac DNA immobilized on nitrocellulose filters. The hybridization mixture contained, in a final volume of 0.5 ml in a scintillation vial, a filter containing 8 μg of Lac DNA, 2.5 μg of ^{3}H-RNA, 4 $\times$ SSC (SSC = 0.15 M NaCl plus 0.015 M sodium citrate), and 0.1% sodium dodecyl sulfate. Non-Lac interactions between labeled RNA and λ DNA were minimized by the addition of 200 μg of unlabeled RNA isolated from the *lac* deletion *E. coli* strain W4032. The vials were incubated at 66 C for 16 to 20 hr. Then the filters were removed, washed with 25 ml of 2 $\times$ SSC, and incubated for 30 min at 37 C in 1 ml of 2 $\times$ SSC containing 20 μg of deoxyribonuclease-free ribonuclease (boiled for 5 min). The filters were then washed on both sides with 100 ml of 2 $\times$ SSC, dried, placed into vials containing Omnifluor, and counted at 40% efficiency in a Beckman LS-100 scintillation spectrometer.

Chemicals. L-Leucine-*U*-^{14}C (255 mCi/mmole), uracil-2-^{14}C (56.3 mCi/mmole), and uracil-6-^3H (25.4 Ci/mmole) were obtained from New England Nuclear Corp., Boston, Mass. IPTG and cyclic AMP were purchased from Calbiochem, Monsey, N.Y.

RESULTS

Capacity of cells induced during inhibition of protein synthesis to form β-galactosidase during recovery. Exponentially growing cultures of *E. coli* B207 (a Rel^+ strain) were exposed to chloramphenicol or puromycin or deprived of K^+ or methionine. In all cases, protein synthesis is inhibited, and stable RNA is made in all situations except during methionine starvation of the Rel^+ strain. IPTG was added to all cultures and incubation was continued. To minimize catabolite repression, which may be severe in nonproliferating cultures incubated in the presence of glucose, the cultures were induced in the presence of cyclic AMP. To assay the capacity of the cells to form β-galactosidase from mRNA templates made during inhibition of protein synthesis, growth was reinitiated at intervals by washing the cells rapidly on membrane filters (Millipore Corp.) and resuspending them in warmed fresh medium lacking the inducer. The presence or absence of cyclic AMP in the recovery medium did not affect the results obtained. This observation is consistent with the finding of Pastan et al. (19, 21) that cyclic AMP acts at the level of transcription.

Practically no enzyme was produced by recovering cells previously induced in the presence of chloramphenicol (Fig. 1A). Cells exposed to chloramphenicol for only 5 min and induced during this time were already incapable of significant synthesis of β-galactosidase after reinitiation of protein synthesis. The same results were obtained with both Rel^+ and Rel^- strains induced in the presence or chloramphenicol (data not shown).

In contrast to this finding, *E. coli* B207 starved of methionine for up to 40 min produced approximately 16 units of β-galactosidase per 10^9 cells during recovery in the absence of inducer (Fig. 1A).

Results obtained with *E. coli* B207 induced during deprivation of K^+ or exposure to puromycin are shown in Fig. 1B and C, respectively. Cells induced during the first 5 min of incubation in the absence of K^+ or in the presence of puromycin synthesized the same amount of β-galactosidase during recovery as the cells starved for methionine. However, the capacity of cells to synthesize β-galactosidase from mRNA made during these treatments decreased with the duration of inhibition of protein synthesis. Cells induced after 35 min of incubation in the absence of K^+ or in the presence of puromycin produced during recovery only about one-tenth of the amount of

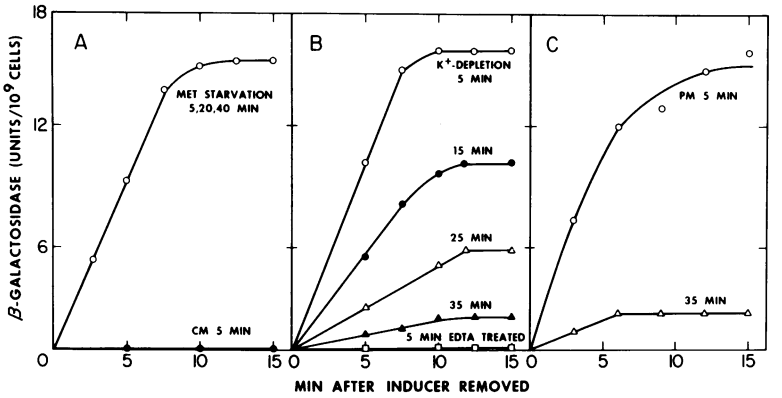

FIG. 1. *Induction of β-galactosidase in the absence of protein synthesis. Growing cells of E. coli strain B207 were rapidly washed by filtration on membrane filters (Millipore; 47-mm diameter, 0.65-μm pore size). The bacteria collected on the filter were washed several times with room temperature basal salts medium and then suspended by placing the filter in the appropriate warmed starvation medium or in growth medium containing chloramphenicol or puromycin. IPTG (10^{-3} M) and cyclic AMP (10^{-3} M) were added and incubation was continued for the indicated times. To reinitiate protein synthesis in these inhibited cultures, the cells were again rapidly washed and suspended in complete growth medium but lacking IPTG and cyclic AMP. Samples were taken at the indicated intervals and the β-galactosidase content of the cells was determined as previously described. Where indicated, cells were treated with EDTA as previously described (13) prior to induction. (A) Chloramphenicol-treated cells (●); methionine-starved cells (○). (B) K⁺-depleted cells. (C) Puromycin-treated cells.*

enzyme formed by cells induced during the first 5 min of protein inhibition.

In some preliminary experiments on the permeability of *E. coli* B207 to cyclic AMP, we observed that even the shortest treatment of cells with ethylenediaminetetraacetic acid (EDTA) had a deleterious effect on subsequent enzyme production. Cells treated with EDTA acid for 30 sec prior to induction during the first 5 min of K^+ starvation failed to produce detectable amounts of β-galactosidase during recovery (Fig. 1B).

Capacity of cells induced during recovery from inhibition of protein synthesis to form β-galactosidase: (i) pulse induction during the first 5 min of recovery. In these experiments we studied the formation of β-galactosidase by cells induced during the first 5 min of recovery from protein synthesis inhibition by chloramphenicol or puromycin or starvation for methionine or K^+. The cells were pulse-induced with IPTG in the presence of cyclic AMP during the first 5 min of reinitiation of protein synthesis, rapidly washed on membrane filters, and resuspended in a fresh, warm growth medium lacking the inducer, and the synthesis of β-galactosidase was determined at the indicated intervals. The results obtained are presented in Fig. 2. The amount of β-galactosidase formed by exponentially growing cells after 5 min of induction is shown as a control in Fig. 2A. Approximately 200 units of enzyme per 10^9 cells are made by cells induced in the presence of cyclic AMP, and about 100 units by cells induced in the absence of cyclic AMP. The addition of cyclic AMP to the cultures during enzyme production had no effect on the amount of β-galactosidase formed. Cells pulse-induced during recovery from 45 min of methionine starvation produced almost as much enzyme as exponentially growing cells (Fig. 2B). Cells induced during recovery after incubation with chloramphenicol were severely impaired in their capacity to form β-galactosidase (Fig. 2B). Cultures incubated with chloramphenicol for 5 min and induced after removal of chloramphenicol produced only about one-tenth of the enzyme compared with that produced by pulse-induced exponentially growing cells or cultures recovering from methionine starvation. The duration of K^+ depletion or incubation with puromycin was the decisive factor which determined the amount of β-galactosidase produced by cells induced during the first 5 min of recovery from these treatments (Fig. 2C and D). Thus, cells of *E. coli* B 207 starved for K^+ or incubated with puromycin for 5 min and pulse-induced during recovery produced the normal amounts of β-galactosidase (about 200 units of enzyme per 10^9 cells). Cells deprived of K^+ for 45 min or incubated with puromycin for 35 min produced only 25 and 20 units of enzyme, respectively, under the above conditions.

(ii) Continuous induction during recovery from inhibition of protein synthesis. Another

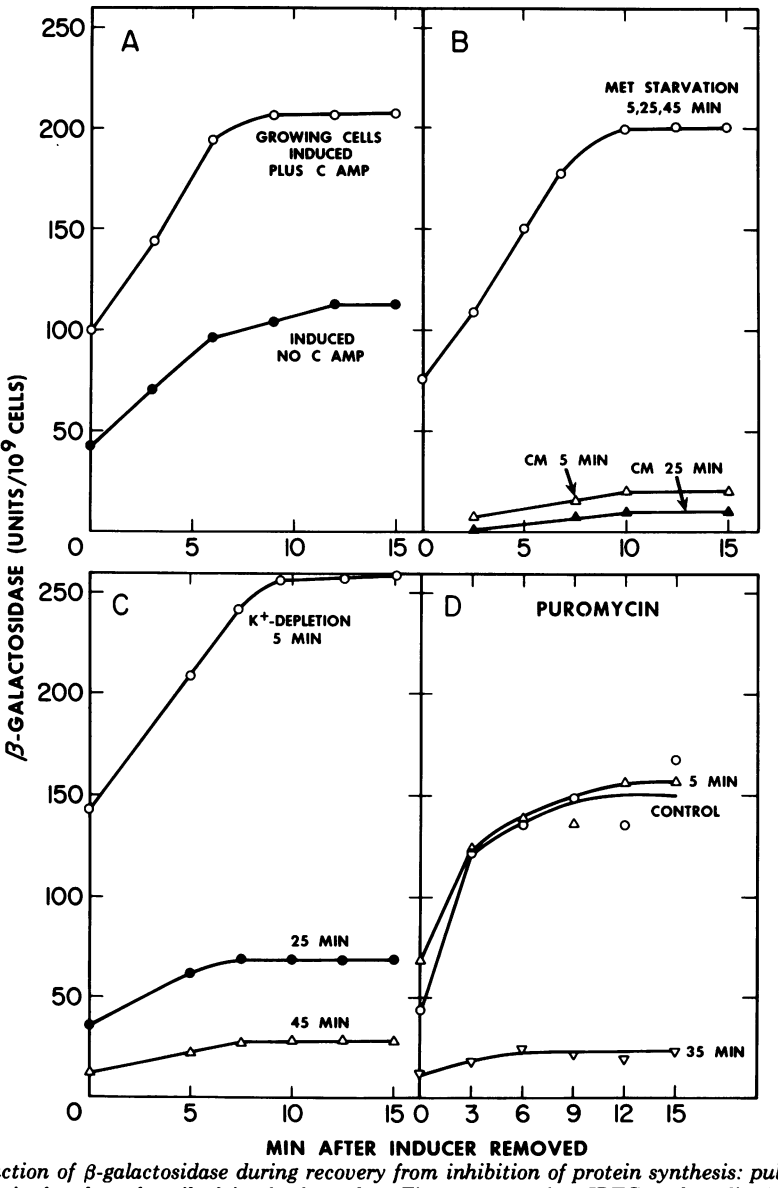

FIG. 2. *Induction of β-galactosidase during recovery from inhibition of protein synthesis: pulse induction. Cells were manipulated as described in the legend to Fig. 1 except that IPTG and cyclic AMP were not present during inhibition of protein synthesis. At the same time as protein synthesis was reinitiated, IPTG and cyclic AMP were added, and incubation was continued for 5 min. Then the cells were rapidly washed on Millipore filters and suspended in fresh warm growth medium lacking IPTG and cyclic AMP. Samples were taken at intervals and the β-galactosidase content of the cells was determined. (A) Growing cultures (as controls) were also induced for 5 min, in the presence and absence of cyclic AMP, and manipulated as described above. (B) Methionine-starved cells (○); chloramphenicol-treated cells (△) and (▲). (C) K⁺-depleted cells. (D) Puromycin-treated cells.*

variation of the experiment described in section (i) was to induce the cells continuously during recovery rather than pulse-inducing. This procedure serves two functions. First, it can be used to determine how long it takes for inhibited cells to recover and start making β-galactosidase at the normal rate. Second, coupled with data on the incorporation of ^{14}C-leucine into protein by these cultures, obtained at the same time, it can be used to determine the differential rate of enzyme synthesis. The results of these experiments are presented in

Fig. 3 and 4. Cells starved of K^+ for 45 min or treated with puromycin for 35 min (Fig. 3C and D) synthesized β-galactosidase initially during recovery very sluggishly compared with cells treated similarly for only 5 min (Fig. 3C and D) or with growing cells (Fig. 3A). Cells treated with chloramphenicol (Fig. 3B) were also impaired in their ability to synthesize β-galactosidase during recovery. In contrast to these findings, Rel^+ cells starved of methionine were unimpaired in their ability to synthesize the enzyme maximally during recovery

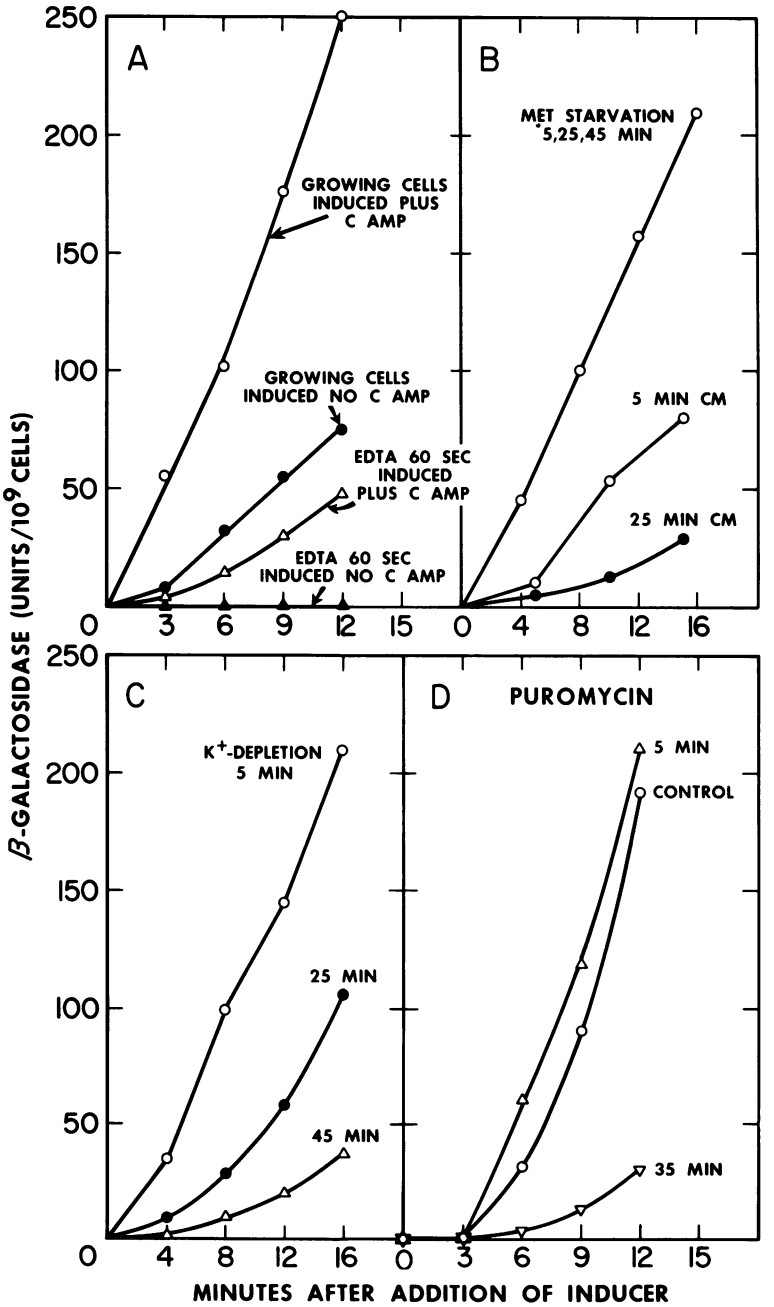

FIG. 3. *Induction of galactosidase during recovery from inhibition of protein synthesis: continuous induction. This experiment was performed as outlined in the legend to Fig. 2 except that IPTG and cyclic AMP were present throughout entire recovery period.*

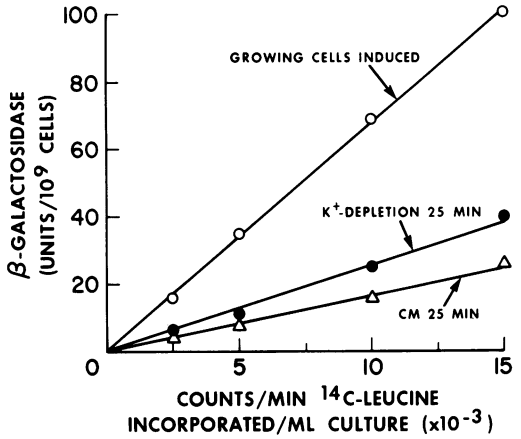

FIG. 4. *Differential rate of β-galactosidase synthesis induced during growth and during recovery from inhibition of protein synthesis. Experiment was performed as outlined in the legend to Fig. 3. Immediately after the cells were suspended in growth medium, ^{14}C-leucine (0.1 μCi/ml; 5 μg/ml) was added to each culture. At the same time as samples were taken for enzyme assay, samples were also taken for determination of incorporation of radioactivity into protein as previously described (2). Synthesis of β-galactosidase in induced growing cultures (○); induced during recovery from 25 min of K⁺ depletion (●); induced during recovery from 25 min of treatment with chloramphenicol (△).*

(Fig. 3B, and Artman and Ennis, *in manuscript*).

Again, the deleterious effect of treatment of growing cells with EDTA prior to induction on subsequent synthesis of β-galactosidase is shown in Fig. 3A.

The observation that β-galactosidase synthesis is inhibited during recovery from K^+ depletion and chloramphenicol treatment could be explained if total protein synthesis was severely inhibited during recovery. If this were so, new enzyme synthesis would be impaired even if the messenger were present, because the messenger could not be translated. Incorporation of ^{14}C-leucine into protein during recovery from the various conditions of inhibition was only slightly or not at all depressed compared with the control untreated cultures. However, as can be seen in Fig. 4, the differential rate of β-galactosidase synthesis (i.e., compared total protein synthesis) was markedly lowered under these conditions. Since we are only looking at short time intervals after recovery, increase in cell number is small and therefore it is not necessary to correct for this in the figures.

Lac mRNA content of cells. To investigate the reason for the decrease in the ability of cells to synthesize β-galactosidase when induced during K^+ starvation or chloramphenicol treatment, or during recovery from prolonged K^+ deprivation or chloramphenicol inhibition, we determined the Lac mRNA content of cells under these conditions. Figure 5 summarizes the results obtained for cells induced during the recovery from inhibition of protein synthesis by chloramphenicol or from K^+ deprivation. As a control, the enzyme levels and Lac mRNA content in growing induced and uninduced cultures are given. The enzyme levels were obtained from the average plateau values from several experiments similar to those described in Fig. 2. Exponentially growing cells pulse-induced for 5 min in the presence of cyclic AMP produced approximately 210 units of enzyme per 10^9 cells, and 0.47% of pulse-labeled RNA was Lac-specific. Uninduced cultures produced little enzyme (less than 1 unit per 10^9 cells) and had little Lac mRNA (0.03% of pulse-labeled RNA).

The amount of β-galactosidase formed and the levels of Lac mRNA in cells induced during recovery from 5 min of K^+ depletion were comparable to those found in control cultures. However, cells induced during reinitiation of protein synthesis after 35 min of K^+ depletion or after 5 min of incubation with chloramphenicol contained an excess of Lac mRNA compared with the amount of enzyme formed. Thus cells induced during recovery from 35 min of K^+ starvation synthesized

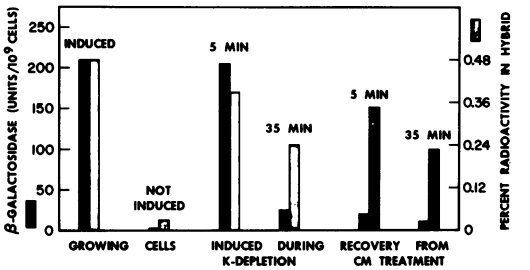

FIG. 5. *Lac mRNA content of growing cells and of cells recovering from inhibition of protein synthesis. The experiments were performed as indicated in the legend to Fig. 2. RNA was isolated from 40 ml of cells pulse-labeled with uracil-6-^{3}H (10 μCi/ml; 25.6 Ci/mmole) from 4 min 15 sec to 5 min and induced during recovery from 5 or 35 min of inhibition of protein synthesis. RNA was also isolated from induced and uninduced cells pulse-labeled for 45 sec during growth. The fraction of Lac-specific RNA was determined as described in Materials and Methods. The data represent averages of 6 to 18 separate determinations on 2 to 6 different preparations of RNA. Enzyme levels were obtained in several experiments similar to those described in Fig. 2.*

about 51% of Lac-specific RNA (0.24% of pulse-labeled RNA) as compared with control cultures but formed only 12% (25 units) of the amount of enzyme. The results obtained with cells induced during recovery after 5 min of incubation with chloramphenicol were even more dramatic. In these cells Lac mRNA comprises 0.36% of pulse-labeled RNA (about 77% of Lac mRNA content in induced growing cells), but only 20 units of enzyme per 10^9 cells (10% of that formed in control cultures) were produced. A similar result was obtained with cells recovering from 35 min of chloramphenicol treatment. These experiments have not yet been performed using puromycin or methionine starvation in this strain.

In cells induced during K^+ starvation or in the presence of chloramphenicol, the levels of Lac mRNA were similar to slightly higher than those found in uninduced growing cultures (0.03%). Cells depleted of K^+ for 5 min contained 0.06% Lac-specific RNA; those depleted for 35 min contained only 0.03%. In agreement with the results obtained by Varmus et al. (21, 22), very little Lac mRNA was found in cells induced for 5 min in the presence of chloramphenicol (0.04%). The amount of enzyme produced by these cells is low (Fig. 1), ranging from 0, in the case of recovery from chloramphenicol inhibition, to about 16 units in cells recovering from 5 min of K^+ depletion.

The fact that cells treated with chloramphenicol or depleted of K^+ were unable to synthesize Lac mRNA when induced during inhibition of protein synthesis could be explained if RNA synthesis during induction were inhibited. That this is not so is shown in Fig. 6 and also has been shown by many previous workers. Incorporation of ^{14}C-uracil into RNA proceeds during inhibition of protein synthesis by chloramphenicol treatment or K^+ depletion. Our results, therefore, cannot be due to an inhibition of RNA synthesis during induction in the absence of protein synthesis.

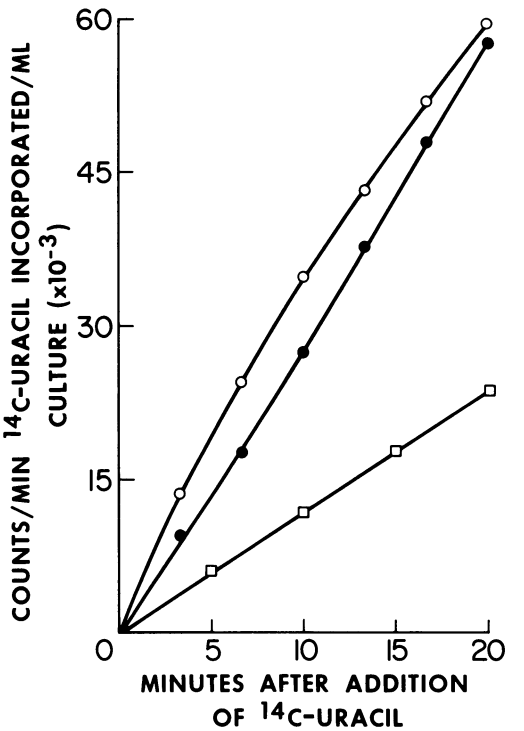

FIG. 6. *Incorporation of ^{14}C-uracil into RNA during inhibition of protein synthesis. Cultures were grown and otherwise manipulated as indicated in the legend to Fig. 1. Immediately after protein synthesis was inhibited, by the methods described in the text, ^{14}C-uracil (0.1 μCi/ml; 2.5 μg/ml) was added, and incorporation into RNA was determined at the indicated intervals as previously described (2). The samples were counted in a Beckman LS-100 scintillation spectrometer at 80% efficiency. Growing cells (●); chloramphenicol-treated cells (○); and K^+-depleted cells (□).*

DISCUSSION

Our present and previous investigations (Artman and Ennis, *in manuscript*) have focused on the regulation of Lac mRNA synthesis and on the coupling of transcription to translation of the *lac* operon in *E. coli*. We have studied the synthesis of Lac mRNA during inhibition of protein synthesis or during recovery from unbalanced growth due to selective inhibition of protein formation, and its translation into functional enzyme. We have purposely chosen four different ways to inhibit protein synthesis because of the different response of the cell to each stress. These are chloramphenicol or puromycin treatment and K^+ or methionine starvation of the appropriate mutants. (i) Chloramphenicol treatment is known to inhibit specifically peptide bond formation (15). Although protein synthesis is rapidly and completely inhibited by high concentrations of the drug, RNA synthesis continues at near the rate observed in uninhibited cells (9). Polysomes are stabilized during chloramphenicol treatment (23). (ii) Puromycin treatment also dissociates RNA from protein synthesis, but its mechanism of action is different from that of chloramphenicol. Puromycin inhibits protein synthesis by forming a peptide bond with peptidyl transfer RNA, and the peptidyl puromycin is released from the polysomes (16). Polysomes break down during

puromycin treatment (7, 20). Unlike the observations of others (22), puromycin at 300 μg/ml completely inhibited incorporation of ^{14}C-amino acid into protein in the strains we used. (iii) Cells of *E. coli* strain B207, which are defective in their ability to accumulate K^+ from the growth medium, do not synthesize protein when deprived of this cation (5). RNA synthesis proceeds during K^+ depletion at about one-half the rate observed during exponential growth. Polysomes are degraded completely during K^+ depletion (4). (iv) Amino acid starvation of Rel^+ strains, in contrast to the above, results in inhibition of both protein and stable RNA synthesis, whereas all of these species of RNA are synthesized in starved Rel^- strains (1, 18).

We have shown that the ability of cells to synthesize Lac mRNA during inhibition of protein synthesis, and to produce Lac mRNA and β-galactosidase during recovery from this inhibition, is dependent on the way protein formation is stopped. There is no obligatory coupling between *lac* operon transcription and its translation.

(i) The production of β-galactosidase from mRNA templates synthesized during methionine starvation or immediately after the addition of the required amino acid to starved cultures of a Rel^- strain of *E. coli* is severely impaired although the levels of Lac mRNA synthesized in these conditions are approximately equal to those found in induced growing cells (Artman and Ennis, *in manuscript*). This observation that mRNA is made but not translated probably explains the observation by Javor et al. (11) of a long lag in induction of β-galactosidase during recovery of Rel^- strains from methionine starvation.

(ii) The amount of β-galactosidase formed by a Rel^+ strain induced during recovery from prolonged methionine starvation was the same as that produced by induced exponentially growing cells (see also Artman and Ennis, *in manuscript*).

(iii) *E. coli* strain B207 fails to synthesize detectable amounts of Lac mRNA during K^+ deprivation and consequently produces very little enzyme after the addition of K^+. A similar result is obtained when puromycin is used to stop protein formation. Cells deprived of K^+ for 35 min and induced during recovery synthesized Lac mRNA in amounts comparable to those found in induced growing cells, but their β-galactosidase synthesizing capacity was severely impaired.

(iv) Cells exposed to chloramphenicol failed to synthesize Lac mRNA during as early as the first 5 min of exposure to the drug. This is in agreement with the results obtained by Varmus et al. (22). Cells exposed to chloramphenicol for 5 min and induced during recovery synthesized β-galactosidase mRNA in amounts comparable to those found in induced exponentially growing cells, but their β-galactosidase synthesizing capacity was severely impaired.

The results showed that Lac mRNA was synthesized by a Rel^- strain during amino acid deprivation, but the Rel^+ strain deprived of K^+ or exposed to chloramphenicol failed to synthesize significant amounts of Lac-specific RNA. These results together with parallel measurements of total mRNA in the same bacterial cultures which showed that under these conditions the inhibited cells synthesized total mRNA at the same rate as growing cultures (Ennis, and Fry and Artman, *unpublished data*) suggest that Lac mRNA synthesis is regulated by a control mechanism distinct from that which regulates total mRNA synthesis.

These results perhaps also indicate that the presence of inducer and cyclic AMP are not the only requirements for Lac mRNA synthesis. Recently Varmus et al. (22) suggested that guanosine tetraphosphate (ppGpp) may be necessary for the synthesis of Lac mRNA, and the low levels of this compound in chloramphenicol-treated cells might be responsible for the failure of cyclic AMP to stimulate the synthesis of Lac mRNA. If this explanation is correct, one should be able to find different ppGpp concentrations in starved Rel^- cells on the one hand and in K^+-deprived and chloramphenicol-treated cultures on the other hand.

Another observation made during these studies is that there is no obligatory connection between transcription of the *lac* operon and its translation. Thus Lac mRNA synthesized by Rel^- strains during amino acid starvation or immediately upon restoration of the required amino acid is not fully translated into functional enzyme (Artman and Ennis, *in manuscript*). Similar results were obtained with cells induced during recovery from incubation with chloramphenicol or from an extended period of K^+ deprivation. Since no impairment of total protein synthesis was found, it would seem, therefore, that the inability of cells to translate Lac mRNA is specific or confined to this messenger and perhaps to few other messengers (inducible ones?). Several explanations to explain the uncoupling of transcription from translation are possible. (i) The translation of Lac mRNA is regulated

by a control mechanism, one of the components of which is either not present during inhibition of protein synthesis or is destroyed under these conditions. (ii) Lac mRNA made under these conditions is not functional because of errors in transcription, or because only a portion of the Z segment is transcribed. (iii) The Lac mRNA formed during recovery cannot be functionally bound to polysomes and consequently cannot be translated. (iv) β-Galactosidase is made from the mRNA synthesized, but it is not enzymatically active. (v) The conditions of stress caused by inhibition of protein synthesis result in an increase of proteolytic enzymes that specifically degrade the β-galactosidase that may be synthesized.

Studies aimed at the elucidation of this problem are presently in progress.

ACKNOWLEDGMENT

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LITERATURE CITED

1. Borek, E., A. Ryan, and J. Rockenbach. 1955. Nucleic acid metabolism in relation to the lysogenic phenomenon. J. Bacteriol. **69**:460–467.
2. Cohen, P. S., and H. L. Ennis. 1965. The requirement for potassium for bacteriophage T4 protein and deoxyribonucleic acid synthesis. Virology **27**:282–289.
3. Davis, B. D., and E. S. Mingioli. 1950. Mutants of Escherichia coli requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
4. Ennis, H. L. 1971. Role of potassium in the regulation of polysome content and protein synthesis in *Escherichia coli*. Arch. Biochem. Biophys. **143**:190–200.
5. Ennis, H. L., and M. Lubin. 1961. Dissociation of RNA and protein synthesis in bacteria deprived of potassium. Biochim. Biophys. Acta **50**:399–402.
6. Ennis, H. L., and M. Lubin. 1965. Pre-ribosomal particles formed in potassium-depleted cells. Biochim. Biophys. Acta **95**:605–623.
7. Ennis, H. L., and B. H. Sells. 1968. Breakdown and reformation of polysomes in *Escherichia coli* during inhibition of protein synthesis. Biochim. Biophys. Acta **161**:503–508.
8. Fry, M., and M. Artman. 1969. Studies of newly synthesized ribosomal ribonucleic acid of *Escherichia coli*. Biochem. J. **115**:295–305.
9. Gale, E. F., and J. B. Folkes. 1953. The assimilation of amino acids by bacteria. II. Actions of antibiotics on nucleic acid and protein synthesis in *Staphylococcus aureus*. Biochem. J. **53**:493–498.
10. Green, M. H. 1966. Inactivation of the prophage lambda repressor without induction. J. Mol. Biol. **16**:134–148.
11. Javor, G. T., A. Ryan, and E. Borek. 1969. Studies of the impaired inducibility in relaxed mutants of *Escherichia coli*. Biochim. Biophys. Acta **190**:442–453.
12. Kepes, A. 1963. Kinetics of induced enzyme synthesis. Determination of the mean life of galactosidase-specific messenger RNA. Biochim. Biophys. Acta **76**:293–309.
13. Leive, L., and V. Kollin. 1967. Controlling EDTA treatment to produce permeable *Escherichia coli* with normal metabolic processes. Biochem. Biophys. Res. Commun. **28**:229–236.
14. Magasanik, B. 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249–254.
15. Monro, R. E., and D. Vazquez. 1967. Ribosome-catalyzed peptidyl transfer: effects of some inhibitors of protein synthesis. J. Mol. Biol. **28**:161–165.
16. Nathans, D. 1964. Puromycin inhibition of protein synthesis: incorporation of puromycin into peptide chains. Proc. Nat. Acad. Sci. U.S.A. **51**:575–582.
17. Pardee, A. B., F. Jacob, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol. **1**:165–178.
18. Pardee, A. B., and L. S. Prestidge. 1956. The dependence of nucleic acid synthesis on the presence of amino acids in *Escherichia coli*. J. Bacteriol. **71**:677–683.
19. Perlman, R. L., and I. Pastan. 1968. Regulation of β-galactosidase synthesis in *Escherichia coli* by cyclic adenosine 3′,5′-monophosphate. J. Biol. Chem. **243**:5420–5427.
20. Ron, E. A., R. E. Kohler, and B. D. Davis. 1966. Increased stability of polysomes in an *Escherichia coli* mutant with relaxed control of RNA synthesis. Proc. Nat. Acad. Sci. U.S.A. **56**:471–475.
21. Varmus, H. E., R. L. Perlman, and I. Pastan. 1970. Regulation of lac messenger ribonucleic acid synthesis by cyclic adenosine 3′,5′-monophosphate. J. Biol. Chem. **245**:2259–2267.
22. Varmus, H. E., R. L. Perlman, and I. Pastan. 1971. Regulation of lac transcription in antibiotic-treated *E. coli*. Nature N. Biol. **230**:41–44.
23. Weber, J., and J. A. DeMoss. 1966. The inhibition by chloramphenicol of nascent protein formation in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. **55**:1224–1230.
24. Yamamoto, K. R., and B. M. Alberts. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale purification. Virology **40**:734–744.