SYNTHESIS OF MESSENGER RNA CODING FOR ELONGATION FACTORS G AND Ts DURING NUTRITIONAL SHIFT-UP IN ESCHERICHIA COLI K-12

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SUMMARY. Following a nutritional shift-up, the rate of ribosomal protein synthesis increased almost immediately, whereas the rates of synthesis of elongation factors G and Ts increased after a lag of 5 - 10 minutes. The messenger RNA content during shift-up was examined using a technique involving labelling of the protein synthesized following inhibition of RNA synthesis. Messenger coding for the ribosomal proteins increased immediately, whereas that coding for the elongation factors G and Ts increased only after a lag of about 10 minutes and closely followed the pattern of messenger coding for total soluble proteins. These data suggest that the observed differences in rates of synthesis of the ribosomal and elongation factor proteins during shift-up are due to differences in the times of transcription of the messengers. The possibility of translational discrimination is discussed.

INTRODUCTION. The genes coding for many components of the protein synthetic machinery including elongation factors G and Tu lie close together in the 64 minutes region of the E. coli chromosome (1,2,3,19). Following an analysis of polar mutants obtained following bacteriophage mμ insertion, Nomura and Engbaek (4) suggested that this region of the chromosome is transcribed as a single unit. This interpretation, however, was called into question by Cabezon et al. (5). Indeed, more recent work by Nomura's group (6) and others (13,14,15) has resulted in the conclusion that the 64 minute region is composed of several transcriptional units. Elongation factor G was not examined in these systems.

Recent studies from this laboratory (8 and this paper) which examined the rates of synthesis of elongation factors G (EF-G) and Ts (EF-Ts) following nutritional shift-up show that formation of these proteins occurred after a lag and therefore was distinct from the observed pattern for the ribosomal proteins. The present studies were designed to determine

whether, following shift-up, (1) the messenger RNA for ribosomal proteins and elongation factors are transcribed simultaneously and translated sequentlially, or whether (2) transcription of these messages occurs in a sequential fashion. To perform these studies, advantage was taken of the drug thiolutin, a reversible inhibitor of RNA synthesis in E. coli.

METHODS

Bacteria and Culture Conditions. E. coli H128 was grown in Cohen's salts medium at 30°C supplemented with acetate (acetate medium) (9). Nutritional shift-up was effected by the addition of a mixture containing glucose, amino acids and nucleosides (enriched medium) to an exponentially growing culture as previously described. (9) Growth was monitored by measuring absorbance at 575 nm. To inhibit RNA synthesis, the drug thiolutin (10,11) was used. Thiolutin has recently been shown to be a specific inhibitor of RNA synthesis at the level of elongation in E. coli (12), and was chosen for the present study because of the rapidity with which it acts and the reversible nature of the block. RNA synthesis in H128 stops completely within 30 seconds of addition of thiolutin to an exponentially growing culture at a final concentration of 10 μg/ml.

Chemicals. (4,5-^{3}H) lysine (30 Ci/mmole) and (U-^{14}C)-Lysine (270 m Ci/mmole) were obtained from New England Nuclear. Protosol and Omnifluor were also obtained from New England Nuclear. Thiolutin was a gift of Nathan Belcher, Pfizer, Inc., Groton, Conn. Solutions of thiolutin were freshly prepared in Cohen's salts at a concentration of 100 μg/ml and used at a final concentration of 10 μg/ml throughout.

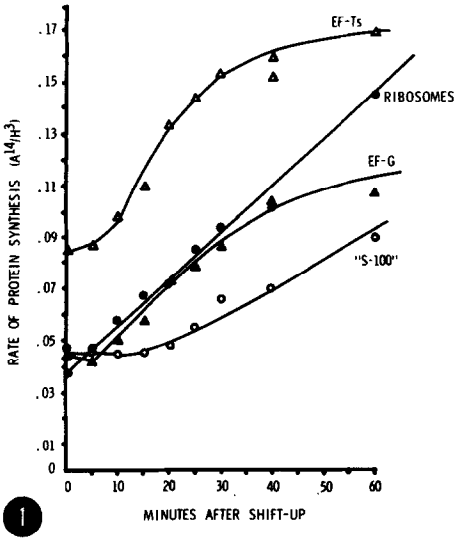
Isolation of Ribosomes, Soluble Protein and Elongation Factors. The disruption of bacteria, separation of total soluble protein S-100 fraction, and preparation of washed 70S ribosomes were performed as previously described (7). The preparation of antibodies specific to EF-G or EF-Ts has been previously described (8). Crude elongation factor preparations were obtained by the addition of $(NH_4)_2SO_4$ to the S-100 fraction to a final

concentration of 65%. The 65% $(NH_4)_2SO_4$ precipitates were collected by centrifugation and resuspended in 1.0 ml 0.05 M/Tris-HCl, pH 7.0, 0.1 M NaCl and 1% glycerol followed by overnite dialysis (1/200) at 4°C against the resuspension buffer. 50 µl of either EF-G or EF-Ts antisera were mixed in the presence of 0.1% Triton X-100 with 100 µl of concentrated crude elongation factor and incubated approximately 18 hrs. at 4°C. The antigen-antibody complexes were washed 2X with resuspension buffer and collected by centrifugation at 15,000 g for 15 minutes.

Analysis of Isotope Content. Aliquots of elongation factor-antibody complexes, S-100 fractions or washed 70S ribosome suspensions were solublized in scintillation vials containing 1 ml of Protosol. Scintillation fluid (0.4% Omnifluor in toluene) was added and the ^3H and ^{14}C content determined. The $^3H/^{14}C$ or $^{14}C/^3H$ ratio of each sample was calculated after the counts were corrected for isotope spillover. Quenching was monitored by external standard ratios.

RESULTS.

Rate of EF-G, EF-Ts, total soluble and ribosomal protein synthesis following shift-up. The rates of synthesis of EF-G and EF-Ts were determined and compared to those obtained for total soluble and total ribosomal proteins. A culture growing exponentially in acetate medium was shifted-up as described in Methods and at intervals thereafter aliquotes were pulse-labelled with ^{14}C-lysine as described in the legend to Fig. 1, and the ribosomal proteins chased into completed ribosome particles. Ribosomes, soluble protein, EF-G and EF-Ts were isolated and their isotope content determined as described in Methods. In Figure 1 the rates of synthesis of these fractions ($^{14}C/^3H$ ratios) are plotted against the time following shift-up. The data indicate that the rate of ribosomal protein synthesis increased almost immediately (lag, less than 5 minutes) whereas increased synthesis of either elongation factor occurred only after 5 - 10 minutes. Total soluble proteins are synthesized at an increased rate following a



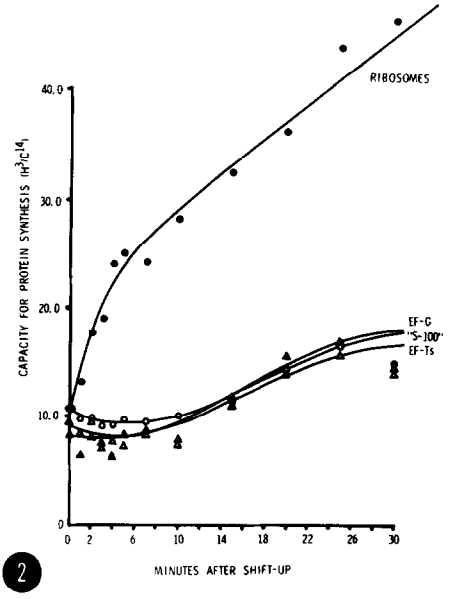


Fig. 1. Rates of synthesis of elongation factors, soluble and ribosomal protein during shift-up. A mid-long phase culture of cells in acetate medium were shifted-up as described in Methods. At the intervals shown, 25 ml aliquots were removed to flasks containing ^{14}C lysine (1 μg and 1 μCi/ml). After one minute, unlabelled lysine was added to a final concentration of 1.5 mg/ml, and the cells incubated for a further 40 minutes. Aliquots of a second culture of cells that had been exposed for several generations to ^{3}H lysine (2 μCi and 10 μg/ml) were added to each flask prior to collection of the cells. Protein fractions were isolated and $^{14}C/^{3}H$ ratios determined as described in Methods.

●—● Ribosomal Protein ○—○ Soluble Protein

▲—▲ EF-G △—△ EF-Ts

Fig.2. Messenger RNA content as a function of time after shift-up. An exponentially growing culture in acetate, pre-labelled for at least 2 generations with ^{14}C lysine, (2 μg and 0.15 μCi/ml) was shifted up as described in Methods. At the intervals shown, samples (18 ml) were removed to flasks containing thiolutin (10 μg/ml final concentration) and ^{3}H-lysine (1 μg and 15 μCi/ml final concentration) and incubation continued for 15 minutes. Unlabelled lysine (1.5 mg/ml) was added, the cells were washed free of thiolutin by centrifugation and washing (2X) in Cohen's salts medium containing excess unlabelled lysine. Ribosomal proteins were chased into completed ribosome particles by incubation in fresh enriched medium containing excess unlabelled lysine for approximately 1 doubling. The soluble protein, washed 70S ribosome and elongation factor fractions were isolated and counted as described in Methods.

●—● washed 70S ribosomes ○—○ soluble proteins (S100)

▲—▲ EF-G △—△ EF-Ts

15 - 20 minute lag. Thus increased elongation factor synthesis took place less rapidly than ribosomal protein synthesis. To determine whether this difference (1) is a reflection of the mRNA's present for these classes of protein, or (2) resulted from a selection of mRNA's for translation, the following experiments were performed.

Synthesis of mRNAs for EF-G, EF-Ts, total soluble and ribosomal proteins during Shift-up. Cultures used in this experiment were pre-labelled with ^{14}C-lysine for at least 2 generations in acetate medium prior to being shifted up as described in Methods. At intervals following shift-up, aliquots were removed to flasks containing ^{3}H-lysine and thiolutin and incubated for 15 minutes by which time protein synthesis had ceased. Excess unlabelled lysine was added and the thiolutin removed. Ribosomal proteins were chased into completed ribosome particles. (For experimental details see the legend to figure 2). The soluble protein fraction, washed 70S ribosomes, and elongation factors were prepared and their isotope content determined as described in Methods. The results are presented in Figure 2 where the capacity for protein synthesis (defined as the total amount of protein that can be synthesized following inhibition of RNA synthesis and used as a measure of the amount of mRNA initially present) is plotted against the time after shift-up. It can be seen that mRNA for ribosomal proteins is present in increasing amount from within the first minute after shift-up. By contrast, soluble protein mRNA actually shows a slight decline before being synthesized in increasing amounts from about ten minutes following shift-up. Strikingly, mRNA coding for the elongation factors closely parallels the soluble protein mRNA. These results clearly show that the regulation of the genes coding for elongation factors is not coordinate with that of ribosomal proteins.

DISCUSSION.

These studies were initiated to determine whether the regulation of the elongation factor genes is coordinate with that of the ribosomal

protein genes, some of which map in the same region of the chromosome as at least the gene for EF-G and one copy of the gene for EF-Tu (1,2,3, 19) (The map location of EF-Ts has not been determined). Nutritional shift-up, during which the ribosomal protein genes undergo a rapid derepression or induction (7 and references cited therein), presents a suitable system in which to examine this question. When the rates of synthesis of these proteins (EF-G, EF-Ts and ribosomal) are examined during shift-up (figure 1) the result suggests a 5-10 minute lag before the appearance of increased EF-G and EF-Ts synthesis as compared to ribosomal protein synthesis which increases immediately. The result is not unambiguous, however, since such a lag could be explained by the delay between translation of proximal and distal cistrons on a very long polycistronic message. It could also be explained by a post-transcriptional processing of a long message followed by more efficient translation of the messengers for ribosomal proteins as compared to the EF messengers.

The existence of a very long transcript for all or part of this region has been ruled out by results from various laboratories (Cabezon et al. (5), Jaskunas et al. (6), Molin et al. (13), Dennis (14) and Kauffmann et al. (15)) in which a variety of techniques and bacterial strains were employed. In recent work from this laboratory (16) where the rates of synthesis of the individual ribosomal proteins and elongation factor protein were determined during nutritional shift-up, it was found that elongation factor protein is synthesized at a much lower rate than any of the individual proteins. These data when considered along with the above studies render unlikely the possibility that the elongation factors can be associated with a polycistronic messenger which also includes ribosomal proteins. However, in a recent paper by Jaskunas et al. (19) it was found that in a transducing phage the copy of the EF-Tu gene mapping at 64 min appears to be part of a transcriptional unit that includes EF-G and ribosomal proteins S7 and S12.

Although the elongation factor gene(s) is probably located on a cistron distinct from these coding for most of the ribosomal proteins, these cistrons could all share the same regulation mechanism. This would imply overall coordinate transcription, followed by discrimination at the translational level, in order to explain the observed difference in the synthesis of ribosomal and elongation factor proteins. (e.g. Fig.1) An attempt was made therefore to analyse the mRNA content during shift-up. The data presented in Figure 2 show very clearly that mRNA transcription for the 2 classes of proteins follows very different patterns during shift-up. These data would appear to rule out the possibility that observed differences in synthesis of the various components of the protein synthetic machinery during shift-up result entirely from discrimination at the translational level and thus suggest that elongation factors and ribosomal proteins are under separate regulatory mechanism.

The interpretation of the data in Figure 2 depends on several assumptions. First, it is assumed that the capacity of the cells for translation during thiolutin treatment is not altered. Control experiments (data not shown) and data published by others (12) in which the effect of thiolutin on the *in vivo* synthesis of β-galactosidase was examined, have failed to demonstrate a direct effect of thiolution on translation. Secondly, it is assumed that mRNA decay rates for a particular species of protein are not changing during shift-up. Coffman *et al.* (17) have reported that the decay rate of β-galactosidase mRNA was invarient over a wide range of growth rates. The capacity for protein synthesis for a single species of mRNA over the duration of this experiment should therefore be a reflection of the amount present. In comparing one species of mRNA with another, the assumption is made that the decay rates do not differ significantly. We have observed that ribosomal protein and total soluble protein messengers do not decay with significantly different rates (to be published). Finally, the assumption is made that the proteins synthesized

during either the pre-labelling or labelling periods are not significantly degraded during the treatment and chase. Studies in which this has been examined have failed to reveal any significant degradation.

In comparing the data in Figure 1, where rates of protein synthesis are presented, with the data in Figure 2 where mRNA content is presented, it can be seen that the messenger for total soluble protein appears to accumulate for 5 to 10 minutes prior to an observed increase in the rate of protein synthesis. This could be evidence for preferential translation of mRNA for r proteins, over mRNA for soluble proteins early during shift-up. That such discrimination may in fact occur is supported by the observations of Dennis and Nomura (18). These authors used an RNA:DNA hybridization technique to examine the mRNA produced during shift-up for a group of approximately 22 ribosomal proteins carried by a λtransducing phage. In comparing the mRNA present with the protein produced, a transient increase in translational yield for ribosomal proteins, occuring between 4 and 6 minutes after shift-up, was observed. Further work will be necessary to clarify this point.

In summary, data are presented which confirm that elongation factor protein synthesis is not coordinated with ribosomal protein synthesis during shift-up. Analysis of mRNA content during shift-up has shown that this is due to differences at the level of transcription. Thus, the production of ribosomal proteins and elongation factors is regulated at the gene level by distinct mechanisms.

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