RIBOSOME PRODUCTION AND ELONGATION FACTOR ACTIVITY DURING A NUTRITIONAL SHIFT-UP IN *ESCHERICHIA COLI*

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1. Introduction

The rate of protein synthesis necessary to support a particular growth rate is thought to depend upon the cellular concentration of ribosomes [1,2]. The initial response of bacterial cells to an enriched nutritional environment leading to an increased growth rate is an immediate increase in the rate of synthesis of ribosomal RNA and ribosomal protein [1,3,4]. Our previous results have shown that the synthesis of ribosomal protein is increased 4-fold during the first 20 min of a nutritional shift-up [5]. The overall rate of protein synthesis, however, does not increase during this period of time. To determine whether the activities of the elongation factors G and T limit the rate of protein synthesis and whether the activities of these factors change co-ordinately with the production of ribosomal protein, we have examined the activities of the elongation factors during a nutritional shift-up.

2. Methods

2.1. *Bacteria and culture conditions*

E. coli H128 was grown as previously described and shifts between media were carried out as previously outlined [5]. *E. coli* D421 TZ, a thymine auxotroph obtained from Dr. H.V. Rickenburg, was used to measure DNA synthesis.

2.2. *Isotopes*

[2-^{14}C]thymidine specific activity 57 mCi/mmole, [^{3}H]L-phenylalanine specific activity 50.5 Ci/mmole, [U ^{14}C]L-lysine specific activity 310 mCi/mmole, and [4,5-^{3}H]L-lysine specific activity 41.6 Ci/mmole were obtained from Schwartz/Mann. [U ^{14}C]L-lysine specific activity 310 Ci/mmole was purchased from ICN. [2-^{14}C]Uracil specific activity 55 mCi/mmole was a product of New England Nuclear. [γ-^{32}P]Guanosine-5'-triphosphate specific activity 2.93 Ci/mmole was purchased from Amersham-Searle. Labelling conditions were as described in the legends.

2.3. *Preparation of extracts*

Bacteria were resuspended in 10 mM Tris–Cl buffer, pH 7.8, containing 10 mM $MgCl_2$ and 1 mM dithiothreitol. The cells were broken by passage through a French pressure cell at 10 000 p.s.i; unbroken cells and cell debris were removed by centrifugation at 20 000 *g* for 15 min. The low-speed supernatant was centrifuged for 427 000 *g* hr to remove ribosomes. High-speed supernatants were quick-frozen in dry ice–methanol and stored at −20°C for no longer than 72 hr. Protein determinations of the extracts were carried out by the method of Lowry et al. [6].

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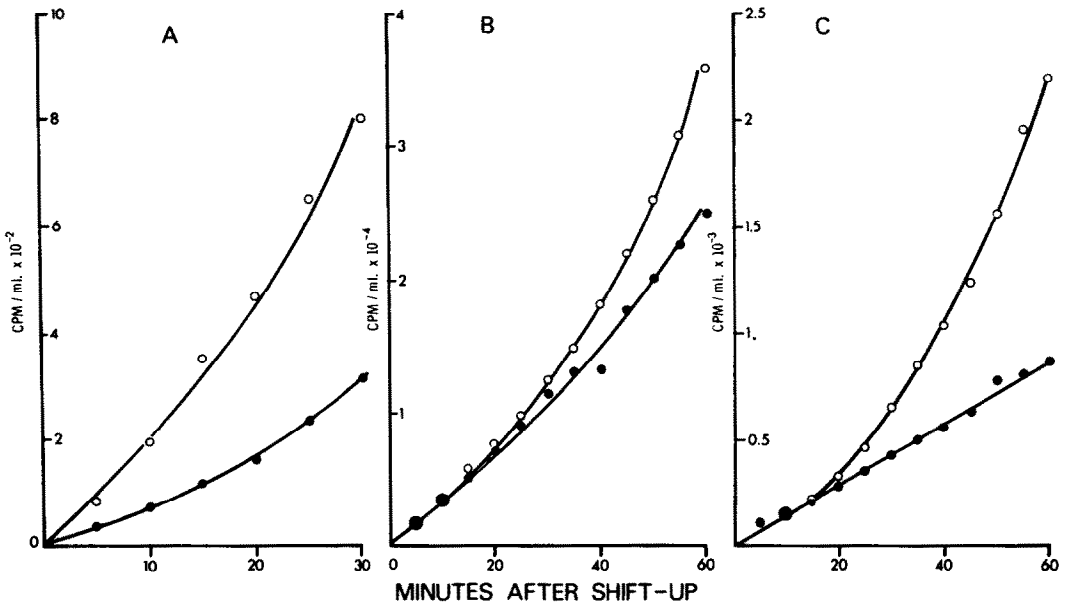

Fig. 1. Macromolecular synthesis during nutritional shift-up. Equal aliquots of cells were transferred from acetate medium into enriched medium (o—o—o) and acetate medium (•—•—•) containing the appropriate radioactive precursor. One ml samples were removed at intervals and added to an equal volume of cold 10% TCA containing an excess of cold precursor. The samples were filtered through millipore discs, washed with cold 5% TCA containing an excess of precursor, dried, dissolved in scintillation fluid and counted. A) RNA synthesis. Cultures contained [^{14}C]uracil (0.10 μCi and 10 μg) per ml. B) Protein synthesis. Cultures contained [^{14}C]leucine (0.066 μCi and 10 μg) per ml. C) DNA synthesis. Cultures contained [^{14}C]thymidine (0.20 μCi and 10 μg) per ml. *E. coli* D421 T2 (thy$^-$) was used.

2.4. *Separation of cellular components*

High-speed supernatants were used as a source of soluble protein. High-speed pellets were resuspended in a small amount of TMA buffer [7] containing 1 mM Mg^{2+} and were used as the ribosome fractions. Ribosome subunits were prepared by layering the ribosome fractions on 5–20% sucrose gradients and centrifuging at 21 500 rpm for 10 hr in an SW 25.1 rotor. Gradients were passed through an ISCO ultraviolet monitor and 1.0 ml fractions were collected. The fractions were assayed for radioactivity by adding 1.0 ml of 10% trichloroacetic acid (TCA) to each fraction and collecting the precipitates on millipore filters. The filters were then washed with 5% TCA, dried and dissolved in a dioxane–naphthalene–omniflour scintillation fluid with 0.5 ml of water.

2.5. *Assays*

Elongation factor G activity in cell extracts was measured as a ribosome-dependent GTPase activity. Reaction conditions were as described by Nishizuka et al. [8]. Specific activity is expressed as cpm of $^{32}P_i$ released from GTP per min of reaction time per μg of protein. Elongation factor T activity was determined by *in vitro* phenylalanine polymerization [8]. Specific activity is expressed as cpm of hot TCA precipitable [^{14}C]phenylalanine per min of reaction time per μg of protein. In crude extracts, the G factor is present in excess thus polymerization is dependent upon the level of the T factor [9].

3. Results

During a nutritional shift-up, an orderly and sequential increase occurs in the rate of synthesis of various macromolecules [10]. Figures 1a, 1b, 1c illustrate the synthesis of RNA, protein, and DNA respectively during the transition from an acetate medium to an enriched medium. While the rate of RNA synthesis increased immediately, the rate of DNA and protein synthesis did not increase until 25 min after the transition.

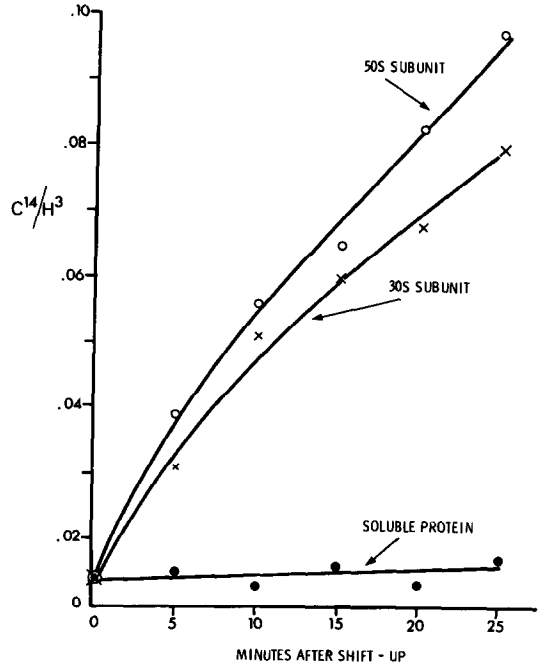

Fig. 2. Synthesis of 50 S, 30 S and soluble protein during a nutritional shift-up. A cell suspension was incubated with [^{3}H]lysine (5 μCi/ml and 10 μg/ml) for 3 to 4 generations in acetate medium. The cells then were transferred to six flasks containing enriched medium (minus lysine). At 0, 5, 10, 15, 20 and 25 min after the shift-up, one of the flasks was selected and [^{14}C]lysine (0.1 μCi/ml and 0.047 μg/ml) was added. One minute later the incorporation of isotope was terminated by adding ^{12}C-lysine (1500 μg/ml). The cells were incubated for an additional 30 min and then were harvested by pouring the suspension over crushed ice and centrifuging as previously described [5]. Radioactivity in the samples was determined by dissolving a small aliquot in 1.0 ml NCS, adding 10 ml of toluene –omniflour scintillation fluid and counting in a Nuclear Chicago scintillation counter.

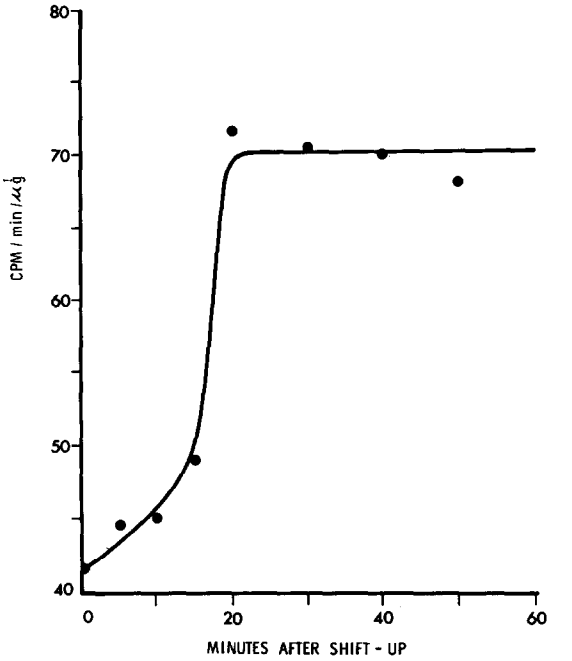

Fig. 3. Specific activity of elongation factor G during a shift-up. At intervals during the shift-up, 50 ml aliquots of cells were removed and chloramphenicol, 100 μg/ml, and 100 g of crushed ice were added. The cells were pelleted and washed once with buffer. Extracts were prepared as described in Methods. Reactions were carried out at 30°C for 5 min with rate limiting amounts of high-speed supernatant.

Our remaining experiments have been designed to determine which component of the protein synthesizing apparatus limits the rate of protein synthesis during the first 20 min of the nutritional shift-up. We have measured the rate of ribosomal protein synthesis during a shift-up. A culture of *E. coli* was incubated for 3 to 4 generations with [^{3}H]lysine during balanced growth in the acetate medium to label all ribosomal proteins in proportion to their concentration. The cells then were pulse-labelled with [^{14}C]lysine for 1 min at various intervals after the shift-up. The pulse was terminated by the addition of a large excess of ^{12}C-lysine and the cells were incubated for a sufficiently long period of time to allow all labelled ribosomal protein to be assembled into mature subunits. Ribosomal subunits and soluble protein were separated and the ^{14}C/^{3}H ratio of each was determined. The rate of synthesis of soluble proteins and ribosomal protein in the subunits during shift-up is presented in fig. 2. These results show that 30 S ribosomal protein apparently is synthesized at a slower rate than 50 S protein during a shift-up. Twenty-five min following shift-up, the ratio of ribosomal protein synthesis to soluble protein synthesis has reached values of 5.8 and 4.7 for the large and small subunits, respectively This ratio is 1.1 when determined during balanced growth in either acetate or enriched medium.

The exaggerated rate of ribosomal protein synthesis during a shift-up is a reflection of the increased rate of formation of ribosome particles. These data agree well with ratios previously determined by another method [5]. Although our previous data

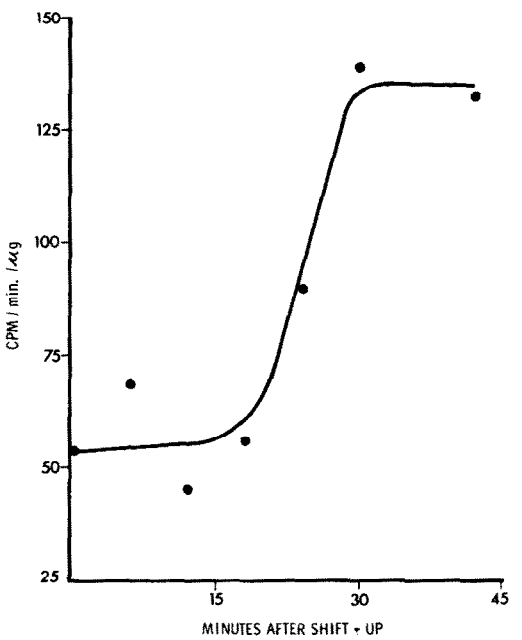

Fig. 4. Specific activity of elongation factor T during a shift-up. At intervals during the shift-up, 50 ml aliquots were removed and added to 100 g of crushed ice. The cells were pelleted and washed once with buffer. Extracts were prepared as described in Methods. Reactions were run at 30°C for 15 min with rate limiting amounts of high-speed supernatant.

showed a small increase in the rate of soluble protein synthesis during the first 25 min of the shift-up, our present results indicate that the rate of synthesis of soluble protein does not increase significantly during this time. We believe that the double-label technique employed in this report is more accurate and reliable than the determination of synthesis in terms of radioactivity per unit of protein. The separation of soluble protein and the ribosomal fraction also was modified in these experiments by increasing the centrifugation time to completely remove ribosomal subunits and precursors from the soluble fraction.

An increase in the number of ribosomes in the cell does not produce an immediate increase in the rate of total protein synthesis during the first 25 min of shift-up. To determine whether the activities of the elongation factors were more closely correlated with the overall rate of protein synthesis, the activities of G and T were measured in crude extracts from cells in balanced growth and undergoing a transition in growth rate. During exponential growth in acetate and enriched media, G factor specific activity was 41.7 and 86.7 respectively, while T factor activity was 30 and 55, respectively. The specific enzyme activities of the G and T factors during a shift-up are presented in fig. 3 and fig. 4, respectively. The data in fig. 3 and 4 demonstrate that the activity of the elongation factor G does not begin to increase until approx. 15 min after the shift-up and reaches its maximum activity by 20 min. The activity of the T factor does not increase until 20 min after the shift-up and reaches its maximum activity by 30 min. These experiments suggest that the synthesis of ribosomal protein and the elongation factors may not be closely co-ordinated. Increases in the specific activity of these enzymes, however, may not reflect an increase in their rate of synthesis because of the possible existence of inhibitors or activators in the extracts. Further experiments to examine the synthesis of the elongation factors directly are now under way. Preliminary results indicate that by 20 min after the shift-up an increase in the rate of synthesis of the elongation factors has occurred.

4. Discussion

The present investigation was initiated to determine how the formation of components of the protein synthesizing system is regulated. Protein synthesizing systems comprise, in addition to ribosomes, a number of protein factors which are involved in the initiation, elongation, and termination of polypeptide chains. Studies of both bacterial [9] and mammalian [12] systems have shown that cells at various growth rates contain a concentration of elongation factors which is stoichiometrically equivalent to the cellular concentration of ribosomes. Thus, rapidly growing cells have high levels of elongation factors and ribosomes while slow growing cells contain lower levels. The stoichiometric correlation between ribosomes and elongation factors has suggested co-ordinate regulation of their synthesis. Data from genetic studies have supported the belief that the *E. coli* G factor may be a member of the same transcriptional unit as a number of ribosomal proteins [13]. Several observations, therefore, have strengthened the idea of co-ordinated synthesis of ribosomal proteins and elongation factors, although this postulate has not been clearly demonstrated.

The nutritional shift-up provides a workable model for examining the concept of co-ordinated control of the synthesis of ribosomes and other components of the protein synthesizing system. Although an increase in the rate of synthesis of ribosome components occurs immediately during a shift-up, the rate of total protein synthesis (fig. 1b) does not increase until 20 min after the shift-up. These observations indicate that the rate of total protein synthesis is not controlled solely by the number of ribosomes in the cell. Recent experiments have shown that the specific activities of aminoacyl tRNA synthetases increase immediately after a shift-up [14]. Consequently, it is unlikely that protein synthesis during a growth rate transition is limited by the availability of charged tRNA. The data in this report indicate that the overall rate of protein synthesis increases 20 min after the shift-up and that the specific activities of the elongation factors increase at approximately the same time. It may be concluded from these studies that the specific activities of the elongation factors may be of prime importance in the regulation of the rate of protein synthesis. Although these results suggest that there is not co-ordinate synthesis of the elongation factors and ribosomal proteins, further studies will be necessary to establish this point.

Studies with eukaryotic systems have suggested that the rate of protein synthesis is regulated by the activity of elongation factors [15–18]. No information is currently available concerning the activity of either initiation or termination factors in these various systems. Thus, control of protein synthesis by these factors cannot be discounted. Nor can regulation by non-protein factors such as MS compounds be exempted. MS compounds have been shown to inhibit elongation factor activities *in vitro* [19–21], but their *in vivo* significance has not been established.

Finally, the results of the present study indicate not only that the ribosomal proteins are synthesized to a greater extent than are the soluble proteins during a shift-up, but also that the ribosomal proteins of the 50 S subunit are synthesized to a greater extent than the proteins of the 30 S subunit. These observations agree with the findings of Michaels [11] who reported that although cells growing in a glucose medium possess an equal number of 50 S and 30 S subunits, cells growing in a poorer medium at a slower growth rate contain an excess of 30 S subunits. Our data indicate that during a transition from a poor to an enriched medium, the imbalance of large and small subunits is readjusted.

Acknowledgements

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