PROTEIN SYNTHESIS DURING A NUTRITIONAL SHIFT-UP IN ESCHERICHIA COLI

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SUMMARY

The rate of synthesis of ribosomal protein increased immediately following a nutritional shift-up in Escherichia coli; while the rate of synthesis of elongation factors did not increase until 5-10 minutes had elapsed. The relative rates of synthesis of EFG and EFTs were constant at all times following shift-up. This constancy was not maintained between elongation factors and ribosomal protein early during shift-up. These data suggest that ribosomal and elongation factor proteins are not co-ordinately synthesized and argue against the postulate that their genes are part of one polycistron.

The studies of Nomura and Engbaek (1) originally suggested that the genetic organization of components of the protein synthesizing system is on one long polycistron. This conclusion was based upon findings using μ phage insertion into the bacterial chromosome. An arrangement was presented indicating that all components proximal to the μ phage insertion were transcribed, while those components located distally to the μ phage insertion were not transcribed. Following a number of insertions, it was concluded that the components of the protein synthesizing system were linearly arranged in one polycistron. More recently, however, Thomas and his colleagues (2) have called this conclusion into question and have indicated that μ phage insertion actually caused gene deletions and that when gene deletions were selected against polar effects were not observed.

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The following studies were initiated to examine the question whether a co-ordinate synthesis of the various components of the protein synthesizing system occurs in such a way as to indicate one polycistron. The relative rates of synthesis of ribosomal proteins and elongation factors were determined in bacterial cells following nutritional shift-up. Previous studies have shown that ribosomal protein and ribosomal RNA synthesis are increased without apparent delay during very early phases following shift-up (3,4). In our earlier reports, the specific enzyme activity of elongation factors G and T did not increase, however, until 15 minutes following shift-up (5). The present investigation was designed to compare the rate of synthesis of ribosomal protein and elongation factors during the early phase of nutritional shift-up and to compare these rates to those obtained during exponential growth.

MATERIALS AND METHODS

E. coli H128 was grown in salts medium at 30°C supplemented with acetate or glucose plus amino acids and nucleosides, (enriched medium) (6). To compare the rate of synthesis of different classes of cellular protein under different growth conditions, a double-label procedure was employed. A suspension of bacteria was labelled with L- (4,5-^{3}H) lysine (2 μC and 10 μg per ml) for 3 to 4 generations of exponential growth. A second suspension of cells (OD_{575} 0.2-0.4) growing exponentially or undergoing a shift-up received L- (U-^{14}C lysine (0.1 μC and 47 ng per ml). Exactly 1.0 min later, an excess of unlabelled lysine was added to arrest isotope incorportation and the cells were incubated for an additional 40 min. to allow assembly of labelled ribosomal protein into mature ribosomal subunits. The ^{14}C and ^{3}H labelled cells were combined, centrifuged and washed with buffer (7).

Disruption of bacteria, separation of ribosomes and soluble protein, and the isolation of ribosomal subunits was performed as previously described (5). Elongation factors were separated from soluble proteins by DEAE cellulose chromatography (8). Both EF G and EF T were eluted from the column in the

same fractions. Ef G was assayed as ribosome-dependent GTP ase activity (9) and was shown to be sensitive to fusidic acid, a known inhibitor of EF G. EF T was assayed by poly U directed polymerization of phenylalanine in the presence of EF G. The elongation factors were further purified by polyacrylamide disc gel electrophoresis (10) and the gels were stained with Coomasie Blue (11). This procedure produced one main protein band on the stained gels which was shown to contain EF G activity. Although Gordon (10) reported that EF G and EF T migrate together under these conditions of electrophoresis, EF T activity was not detected in our gels. This may be due to heat generated during electrophoresis or the presence of persulphate in the gels. The band in the gels containing EF G, therefore, may also contain EF T.

Antibodies against EF G and EF Ts were prepared in New Zealand white rabits (about 1-2 kg) by intramuscular injections of 0.5-1 mg of antigen emulsified in an equal volume of complete Freund's adjuvant. After 3 weeks, the animals were given booster shots of 0.25-5 mg of antigen in an equal volume of incomplete Freund's adjuvant. After 2 weeks, the animals were bled from the ear and serum collected by centrifugation after allowing the blood to clot. Merthiolate (0.01%) was added to the serum which was then stored at $4^{o}C$. Ouchterlony plates were poured using 1% agarose in 0.05 M Tris, pH 7.5, 0.1 M NaCl, 1 mM EDTA. Each S-100 fraction was assayed in a separate plate by adding 50 µl of antiserum to a center well and 50 µl of S-100 to peripheral wells 4 mm from either side of the center well. The plates were incubated overnite at $23^{o}C$ and then dialyzed extensively for 2 days against phosphate buffered saline. The immunoprecipitin bands were punched out, incubated overnite in 1.0 ml of protosol at 50^{o} C and the C^{14} to H^{3} ratio in the EF G and EF Ts band deter mined by scintillation counting. Background radioactivity was determined by punching out areas of the Ouchterlony plates not having any immunoprecipipitin bands. Attempts to prepare antibody against EFTu were unsuccessful.

The isotope content of the total cellular protein was measured by withdrawing an aliquot of the broken cell preparation prior to fractionation. The

sample was solubilized in a scintillation vial containing 1 ml of Protosol. Ten ml. of scintillation fluid (0.4% Omnifluor to toluene) was then added. The isotope content of soluble protein or ribosomal protein following fractionation was determined in the same manner. The radioactivity in the gel band containing elongation factor protein was determined by slicing the band from the gel and placing it in a scintillation vial containing 10 ml. of scintillation fluid (0.4% Omnifluor and 3% Protosol in toluene). The vial was tightly capped, and incubated at 37° for 24 hours. The H^3 and C^{14} content of all samples was measured in a Beckman scintillation counter. The C^{14}/H^3 ratio of each sample was calculated after the counts were corrected for isotope spillover. Quenching was monitored by external standard ratios.

RESULTS AND DISCUSSION

The first experiment performed was designed to determine the rates of synthesis of total cellular protein, ribosomal protein and elongation factor protein during the early phases of nutritional shift-up. Suspensions of bacterial cells (25 ml. each) prelabelled with H^3-lysine during growth in

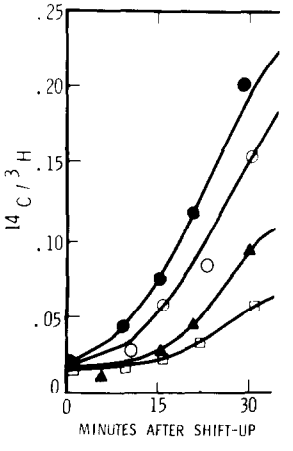

Fig. 1. Synthesis of cellular protein for 30 min. following shift-up. Cells were prelabelled with ^{3}H-lysine in acetate medium and with ^{14}C-lysine after transfer to enriched medium for 1 min. as described in Methods. The $^{14}C/^{3}H$ ratios of total cell protein, □—□, elongation factor protein, ▲——▲, 30S ribosomal protein, ○——○, and 50S ribosomal protein, ●——●, were determined as described in Methods.

acetate medium were transferred to each of twelve flasks containing enriched medium (shift-up). At predetermined times after the shift-up, cells were incubated with C^{14}-lysine for one minute and processed as described in the methods section. The data presented in Figure 1, illustrate the C^{14} to H^3 ratio of total cell protein, 30S and 50S ribosomal protein and elongation factor protein determined during the first 30 minutes following shift-up. These results indicate that there is an immediate increase in the rate of ribosomal protein synthesis. Synthesis of total cell protein and elongation factor protein increased after a fifteen minute delay. Making use of these data, a table is constructed in which the rate of ribosomal protein synthesis and the rates of elongation factor synthesis are expressed as a function of total protein synthesis. Presenting the data in this way eliminates any influence changes in amino acid pool size might cause. The data, in Table I, indicate that at 0 time following nutritional shift-up, the rates of ribosomal

TABLE I

Differential Rates of Synthesis

of Ribosomal Proteins and Elongation Factors

Time after shift-up	$\frac{C^{14}/H^3 \text{ Ribosomal Protein}}{C^{14}/H^3 \text{ Total Protein}}$		$\frac{C^{14}/H^3 \text{ Elongation Factors}}{C^{14}/H^3 \text{ Total Protein}}$
	50S	30S	
0	$\frac{.016}{.015} = 1.07$	$\frac{.016}{.015} = 1.07$	$\frac{.014}{.015} = .93$
10	$\frac{.045}{.016} = 2.81$	$\frac{.025}{.016} = 1.56$	$\frac{.016}{.016} = 1.00$
15	$\frac{.074}{.020} = 3.70$	$\frac{.060}{.020} = 3.00$	$\frac{.026}{.020} = 1.30$
30	$\frac{.194}{.055} = 3.57$	$\frac{.153}{.055} = 2.78$	$\frac{.093}{.055} = 1.69$

protein synthesis and elongation factor synthesis expressed as a function of soluble protein formation is essentially equivalent. At later times (15 and 30 minutes) following shift-up, however, the relative rates of synthesis of ribosomal and elongation factor protein versus total protein show a marked variation from 1. These data reveal that the rates of synthesis of the ribosomal protein change to a much greater extent than the rates of elongation factor synthesis.

To record more precisely the initial cellular response to shift-up, the experiments were repeated and in this case nutritional supplements were added directly to cells growing in acetate medium and measurements made at shorter intervals following shift-up. Equal portions of a suspension of E. coli growing exponentially in acetate medium were placed in five flasks. When the cells reached the mid log phase of growth, a concentrated mixture of nutritional supplements were added to each flask. The final concentration of these supplements was exactly as described for the enriched medium. At the times indicated in Figure 2, one of the flasks was selected and C^{14}-lysine added. Incorporation of the isotope was terminated after one minute by the addition of unlabelled lysine and the cells were incubated for an additional forty-five minutes, to allow all the ribosomal protein synthesized to be assembled into ribosome particles. The cells were poured over crushed ice and an equal volume of cells labelled with H^3-lysine during growth in acetate medium was added to each sample of C^{14}-labelled cells. The double labelled cells were centrifuged and the C^{14} to H^3 ratios of total cell protein, elongation factor protein and ribosomal protein determined. The results in Figure 2 show that the rate of total cell protein synthesis remains constant during the first ten minutes. A change in the rate of elongation factor protein synthesis was observed by six minutes and by ten minutes had increased by 30%. The rates of 30S and 50S ribosomal protein synthesis increased 10% and 30% respectively during the first two minutes of the shift-up. By ten minutes following the shift-up, the rates of synthesis of 30S and 50S ribosomal protein had at

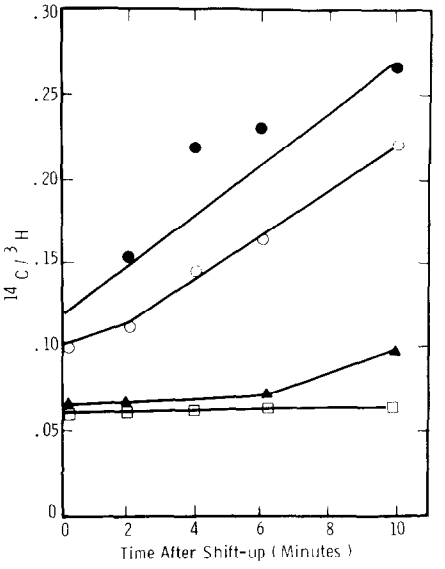

Fig. 2. Synthesis of cellular protein during the first 10 min. of a shift-up. Cells were labelled with ^{3}H-lysine in acetate medium and ^{14}C-lysine after transfer to enriched medium as described in text. The $^{14}C/^{3}H$ ratios of total cell protein, □——□, elongation factor protein, ▲——▲, 30S ribosomal protein, ○——○, and 50S ribosomal protein, ●——●, were described in Methods.

least doubled. Although ribosomes and elongation factor proteins are physiologically interrelated and are present in equimolar amounts during exponential growth, these experiments suggest that they are not synthesized in a co-ordinate manner during a shift-up. The data presented in Table 1 supports this contention.

Since the methods used to isolate elongation factors shown in Figure 2 did not separate EF G and EF T, antibodies to EF G and EF Ts were prepared. Cells previously grown in presence of H^{3}-lysine were pulse-labelled with C^{14}-lysine (1.0 μc/ml, 470 ng/ml) at various intervals following shift-up and the soluble protein (S-100) fraction separated from the disrupted bacteria as described in the Methods section. The S-100 fractions were then analyzed using double diffusion analysis on Ouchterlony plates. The immunoprecipitin bands were excised and the C^{14} to H^{3} ratio in the EF G and EF Ts bands

determined. The results from this experiment are shown as in Table 2 and demonstrate the change in the rate of synthesis of EF G or EF Ts relative to total protein. Both EF G and EF Ts demonstrate a 5 minute lag before significant increases occur in their rate of synthesis. These data substantiate the result in Table 1 regarding the increase in the rate of synthesis of the elongation factors following shift-up; the rate of increase of EF synthesis is much slower than the rate of increase in ribosomal protein (see Figure 1). The C^{14} to H^3 ratios of the individual ribosomal proteins described for Figure 2 have been reported elsewhere and show that elongation factor protein is synthesized at a much lower rate than any of the individual ribosomal proteins during nutritional shift-up (12).

The data presented in these studies support the conclusion of Thomas and his colleagues and suggests that ribosomal proteins and elongation factors are not part of one polycistron. It is interesting to note following shift-up that

TABLE 2

Differential Rates of Synthesis of EF G and EF Ts

Time after shift-up	$\frac{C^{14}/H^3 \text{ in EF G}}{C^{14}/H^3 \text{ in total protein}}$	$\frac{C^{14}/H^3 \text{ in EF Ts}}{C^{14}/H^3 \text{ in total protein}}$
0	$\frac{0.045}{0.047} = 0.96$	$\frac{0.085}{0.047} = 1.81$
5	$\frac{0.043}{0.046} = 0.94$	$\frac{0.088}{0.046} = 1.91$
10	$\frac{0.051}{0.045} = 1.13$	$\frac{0.098}{0.045} = 2.18$
15	$\frac{0.058}{0.046} = 1.26$	$\frac{0.11}{0.046} = 2.39$
20	$\frac{0.073}{0.049} = 1.49$	$\frac{0.134}{0.049} = 2.74$
30	$\frac{0.088}{0.060} = 1.46$	$\frac{0.153}{0.060} = 2.55$

the relative rates of synthesis of the two elongation factors are constant. The same constancy is not maintained, however, between elongation factors and ribosomal proteins through the early periods of shift-up.

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