AMOUNTS OF FREE 70S RIBOSOMES AND RIBOSOMAL SUBUNITS FOUND IN ESCHERICHIA COLI AT VARIOUS TEMPERATURES

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SUMMARY

When the rate of protein synthesis in cultures of Escherichia coli was decreased by lowering the temperature, free 70S ribosomes accumulated and the number of polysomes per cell decreased, while 50S and 30S ribosomal subunits remained almost constant.

INTRODUCTION

Recent studies have shown that 70S ribosomes in Escherichia coli cells are released as such from messenger RNA on completion of polypeptide chain synthesis and that interaction of a dissociation factor (1) with 30S subunit of a 70S ribosome causes the ribosomes to dissociate into subunits(2). The products of polysome run-off accumulate as free 70S ribosomes, which are free of peptidyl-tRNA and messenger RNA, after treatment with actinomycin D or with puromycin, or following starvation for a carbon source or for certain amino acids(3,4,5,6), while the level of ribosomal subunits remains essentially constant.

We have now determined that cells grown at low temperatures behave similarly even in the presence of excess nutrient. Once again, free 70S ribosomes accumulate at the expense of polysomes while the ribosomal subunits remain almost constant.

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MATERIALS AND METHODS

Escherichia coli K12 w3110 strain was used. Cultures were grown on a rotary shaker, at various temperatures in medium containing 0.2% casamino acids(Difco) and 0.4% glucose as carbon source(7).

50 ml cultures in exponential growth were supplemented with 5 μM uracil containing 10 to 15 μc ^{3}H-uracil(Daiichi Kagaku Co Ltd) and were incubated at 37°, 23.5°, 14°and 8.5°C for 3 to 18 hours. The cultures were then rapidly poured over an equal volume of crushed ice containing chloramphenicol(200 μg/ml). Spheroplasts were formed at 0°C on addition of a final concentration of 200 μg lysozyme/ml and 2.5 mM EDTA and lysed by a modification of the method of Flessel et al(8,9). To the collected spheroplasts was added 1 ml of lysing medium consisting of Tris-Mg buffer(0.01M-Tris; pH 7.5 in 0.01M $MgCl_2$) containing Brij 58 (0.4%), sodium deoxycholate(0.25%), chloramphenicol(100 μg), KCl(60 mM) and DNase 10 μg/ml(Worthington RNase free). The cell-lysates were layered on 23.5 ml of 10 to 30% linear sucrose gradient in 0.01M Tris-0.01M $MgCl_2$-0.06M KCl(pH 7.5). 7.5 ml of 40 to 70% sucrose in the same buffer was placed in the bottom of the tube. After centrifugation at 25,000 rpm for 195 min in the SW 25.1 rotor of a Beckman L2 at 4°C, 20 drop fractions were collected from the bottom of the tube. Aliquots of each fraction were used for determination of the absorption at 260 mμ and the radioactivity. The radioactivity was measured in a Beckman, Ls 200B, liquid scintillation system.

RESULTS AND DISCUSSION

1. Analysis of polysomes, free 70S ribosomes, 50S and 30S subunits of lysate obtained from Escherichia coli cells cultured at 8.5°C.

An exponentially growing culture was incubated with ^{14}C-tyrosine (0.4 μc/ml) at 8.5°C for 20 min. A cell-lysate was prepared and analysed as described in Materials and Methods.

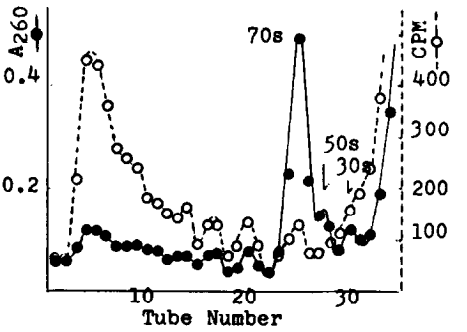

Fig 1 Sedimentation analysis of polysomes, free 70S ribosomes, 50S and 30S subunits from cells cultured at 8.5°C.

E.coli cells were cultured at 8.5°C for 14 hours, and then were incubated with ^{14}C-tyrosine(Daiichi kagaku specific activity 322 mc/mM) for 20 min. A cell-lysate was prepared from the labeled cells and layered on a sucrose gradient as described in Materials and Methods. After centrifugation at 25,000rpm for 195 min in the SW 25.1 rotor, materials were collected in 20 drops from the bottom of the tube. Aliquots of each fraction were used for determination of absorption at 260 mμ and radioactivity. Each fraction was precipitated with an equal volume of 10% trichloroacetic acid. The resulting precipitates were washed with hot 5% trichloroacetic acid. The radioactivity was measured as described in Materials and Methods.

As seen in Fig 1, a polysomal region was found in tubes 1 to 21 and a region of 70S ribosomes and the subunits was found in tubes 22 to 31.

The cell-lysate gave a prominent peak of absorbance at 260 mμ in tube 25, while the radioactivity, found in a separate experiment to be almost completely chasable with excess unlabeled L-tyrosine, was detected in the polysomal region and in tube 25 of the 70S ribosomal region. The specific activity(CPM/$A_{260m\mu}$) in the 70S position is much less than that found in the polysomal region. From these observations, the large amount of 70S ribosomes presumably represents free 70S ribosomes and not a degradation product of polysomes during polysome preparation. Fig 1 also shows that the broad peak of absorbance at 260 mμ which was seen in tubes 1 to 11 had a prominent peak of radioactivity. Following treatment with 5 μg/ml of RNase

(Worthington) at 4°C for 10 min, the radioactivity in tubes 1 to 11 was completely shifted to the 70S position, while absorbance at 260 mμ in the tubes partially disappeared. It seems likely that this broad peak(tubes 1 to 11) with an average $A_{280m\mu}/A_{260m\mu}$ of 0.7 to 0.75 contains membrane debris as well as polysomes. Therefore a absorbance at 260 mμ may not always be an accurate measure of the polysomes in tubes 1 to 11. The true number of polysomes in this region is indicated by ^{3}H-uracil labeled ribosomal RNA.

2. Sedimentation profiles of lysates obtained from cells cultured at various temperatures.

Cultures were incubated with ^{3}H-uracil at 37°C for 1 hour and then divided into four parts. Incubation was continued in each case at 8.5°, 14°, 23.5°and 37°C for 18, 16, 14 and 2 hours respectively in the presence of ^{3}H-uracil. Lysates were prepared from each cell suspension and analysed as described in Materials and Methods. As seen in Fig 2, the radioactivity profiles showed that lysates from cells grown at 23.5° and 37°C contained a large number of polysomes, while those from cells grown at 8.5°and 14°C showed a preponderance of free 70S ribosomes.

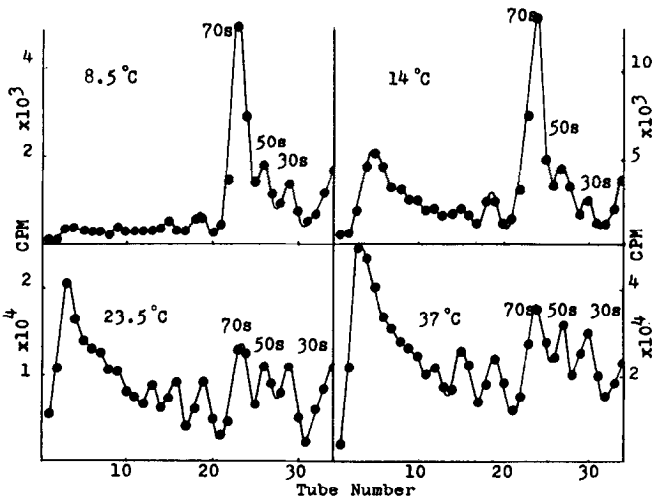

Fig 2 Sedimentation radioactivity profiles of lysates obtained from cells cultured in the presence of ^{3}H-uracil at various temperatures.

From these data, the relative amount of polysomes was determined, and as shown in Fig 3, polysomes were markedly decreased in the cells grown at low temperature.

3. Relative amount of free 70S ribosomes and subunits.

In order to clarify the distribution patterns of free 70S ribosomes, 50S and 30S subunits, each lysate from cells cultured at various temperatures was layered on 5 to 20% sucrose and centrifuged at 25,000 rpm for 4 hours. In the distribution patterns of the cell-lysates, at 8.5°, 14°, 23.5°and 37°C, 62%, 43%, 40% and 38% respectively of non-polysomal ribonucleoproteins, which were composed of free 70S ribosomes and the ribosomal subunits, were in the form of 70S ribosomes. These 70S ribosomes were dissociated into 50S and 30S subunits in 1 mM Mg^{2+}. Intermediate 60S particles such as reported by Schreier et al(10) were not observed.

It can be seen from Fig 3 that content of free 70S ribosomes was markedly increased at low temperatures. Thus, 43% of the total ribosomes were present as free 70S ribosomes in lysate of cells grown at 8.5°C whereas only 12% were in this form in lysate of cells grown at 37°C . On the other hand, the amount of 50S and 30S subunits remained almost constant between 25 and 20% at various temperatures (various rate of protein synthesis: Fig3) tested.

We conclude that when the rate of protein synthesis in cultures of E.coli is decreased by lowering the temperature, ribosomes are stored mainly in the form of 70S ribosomes, and dissociation of 70S ribosomes into subunits occurs when cells are actively synthesising proteins.

Fiedman et al(11) reported that lowering the temperature of E.coli to 8°C results in an accumulation of ribosomal subunits due to a block in the initiation of protein synthesis. In our laboratory when cell-lysate were prepared from cells incubated at 8.5°C for 1 to 2 hours and using a lysing medium containing 2.7 μg/ml RNase, such a large amount of subunits was not seen.

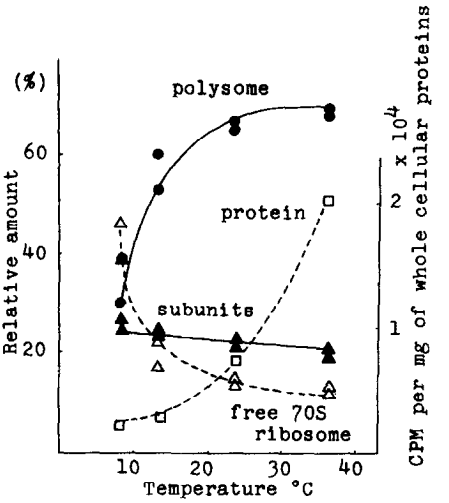

Fig 3 Changes of relative amount of polysomes, free 70S ribosomes and the subunits in sedimentation patterns of cell-lysates obtained from cells cultured at various temperatures, and rate of incorporation of ^{14}C-leucine into whole cellular proteins at various temperatures.

Analyses of polysomes, free 70S ribosomes and the subunits of cells cultured at 8.5°, 14°, 23.5°and 37°C were done in duplicate as described in the text and Materials and Methods, and then from the sedimentation patterns, the percentages of various forms of ribosomes were calculated. Polysome(—●—), free 70S ribosomes(--△--), subunits (—▲— 50S and 30S ribosomal subunits).

Cells were cultured at 8.5°, 14°, 23.5°and 37°C for 10 hours and then incubated with 0.2 μc/ml of ^{14}C-leucine(Daiichi kagaku specific activity 162 mc/mM) for 30 min. The cultures were precipitated with an equal volume of 10% trichloroactic acid. The resulting precipitates were washed with cold and hot trichloroacetic acid, and counted as described in Materials and Methods. CPM per mg of whole cellular proteins(--□--). Protein was determined by the methods of Lowry et al.

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