POLYPEPTIDE SYNTHESIS IN *ESCHERICHIA COLI* EXTRACTS: EFFECT OF SPERMIDINE ON THE EXCHANGE OF RIBOSOMAL SUBUNITS

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1. Introduction

The ribosome cycle has been intensively studied in bacteria, both *in vivo* and with cell-free systems. However, several aspects are not yet clear, and conflicting interpretations on the kind of ribosomes (either subunits or 70 S particles) liberated from polysomes upon termination of translation have been reported.

The physiological significance of free monomers is also a matter of discussion even though their existence has now been generally accepted.

In the last few years the exchange of ribosomal subparticles between the ribosomes participating in protein synthesis and the pool of free subunits has been demonstrated [1–3]. From these studies several investigators have concluded that on completion of polypeptide chains the 30 S and 50 S particles are released from mRNA and immediately join the pool of subunits with which they can freely exchange.

On the other hand it has been shown more recently that the exchange of subparticles can also occur after termination of translation [4, 5], and therefore it can be the result of a subsequent step of dynamic equilibrium between run-off 70 S monomers and ribosomal subunits.

In this report we describe some studies on polypeptide synthesis in a cell-free system from *E. coli* and the exchange of subunits under several conditions. Our aim was to discern whether the exchange takes place simultaneously with or after the completion of polypeptide chains. We have looked for a stabilizing agent of 70 S monomers in order to block any exchange subsequent to the formation of these particles. After trying several substances as neomycin, methanol and ethanol, all of which partially inhibited protein synthesis, we have found that spermidine can accomplish the required function. This polyamine, normally present in bacterial extracts, has a strong stabilizing effect on 30 S–50 S couples [6] without affecting the polypeptide synthesis in our conditions.

2. Materials and methods

E. coli D_{10} was grown as described previously [7], except that the medium contained Casamino acids (2 g/l) instead of a complete mixture of amino acids.

The harvesting of bacteria after fast cooling of cultures in the early exponential phase and the preparation of lysates have been already reported [7].

The purified polyribosomes, S_{100} supernatant fluid free of subunits and ^{32}P-labeled 30 S particles were obtained as will be described elsewhere [8].

Spermidine phosphate was purchased from Mann and ^{14}C-amino acids (reconstituted protein hydrolysate) from Schwarz BioResearch.

Standard assay: Each 0.125 ml reaction mixture contained 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer of pH 7.4, 8 mM magnesium acetate, 64 mM KCl, 1.4 mM ATP, 0.3 mM GTP, 8 mM P-enolpyruvate, 16 mM 2-mercaptoethanol, a complete amino acid mixture (0.1 mM each), S_{100} supernatant fluid (0.1–0.4 mg of protein)

and 0.5 to 3.0 A_{260} units of purified polyribosomes. Where indicated a spermidine solution at pH 6.5, ^{14}C-amino acids or ^{32}P-labeled 30 S particles were added. The addition of pyruvate kinase did not increase the polypeptide synthesis, suggesting that the supernatant fluid has a sufficient amount of this enzyme.

The incubations were carried out at 37° and the tubes were then chilled to 0°. In order to determine the exchange of radioactivity between 30 S and 70 S particles the samples were layered on 12 ml of 5–20% linear sucrose density gradients made up in 20 mM Tris-HCl buffer, pH 7.8, 10 mM magnesium acetate, 50 mM KCl, and centrifuged for 75 min at 35,000 rpm in a Spinco SW 40 rotor at 3°.

The absorbance at 254 nm was continuously recorded with an Isco ultraviolet analyzer; the fractions of the gradients were collected into vials and counted after the addition of Bray's solution.

The amino acid incorporation was measured after precipitation with 5% trichloroacetic acid, heating at 90° for 15 min and filtration through Millipore filters.

All radioactivity determinations were performed with a Packard TriCarb liquid scintillation spectrometer.

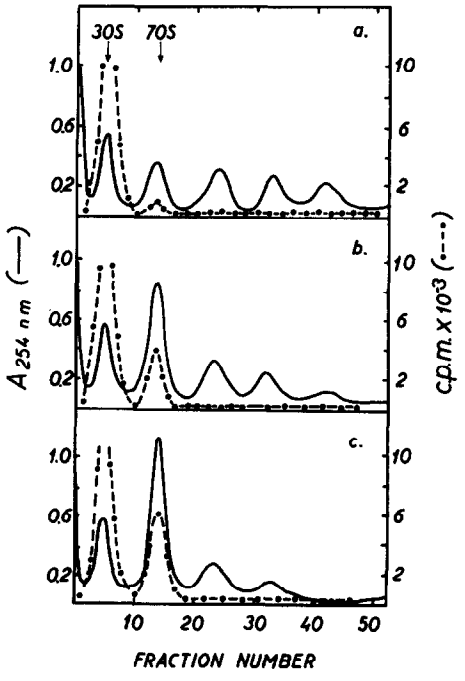

Fig. 2. Ribosomal distribution patterns and exchange of subunits after different times of polypeptide synthesis. Conditions and reaction mixture as in methods with the addition of 0.3 A_{260} units of ^{32}P-labeled 30 S subunits (100,000 cpm/A_{260} unit). a) 0 min; b) 10 min and c) 40 min incubation.

3. Results and discussion

Fig. 1 illustrates the kinetics of polypeptide synthesis in the cell-free system with endogenous mRNA.

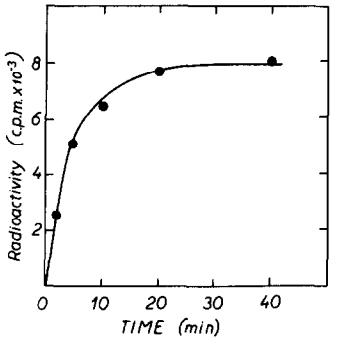

Fig. 1. Kinetics of amino acid incorporation in the *E. coli* cell-free system. The assay conditions and reaction mixture were as described in methods with the addition of 0.05 μCi of ^{14}C-amino acid mixture.

The amino acid incorporation increased linearly at the beginning of the reaction. It leveled off after 5 min and reached a plateau at about 20 min of incubation. The addition of purified 30 S subunits and/or 1 mM spermidine affected neither the amount of amino acid incorporated into the hot trichloroacetic acid insoluble material nor the completion and release of polypeptide chains [8].

Several investigations on the exchange between "heavy" and "light" particles during protein synthesis have shown that some artifacts can affect the results. In these experiments it could be very difficult to distinguish hybrid ribosomes of intermediate sedimentation constant from "heavy" particles partially dissociated during the centrifugation in sucrose gradients [5]. For this reason we have followed the Falvey and Staehelin's approach [9], measuring the exchange between 70 S monomers and the radioactive smaller subunits. Our *in vitro* system contained polyribosomes

Table 1
Effect of sparsomycin on the exchange of subunits.

Additions during first incubation	Additions during second incubation	Exchange (%)
^{32}P-30 S	–	22.1
^{32}P-30 S + sparsomycin	–	4.8
–	^{32}P-30 S	25.4
–	^{32}P-30 S + sparsomycin	24.1

0.3 A_{260} units of radioactive 30 S particles and/or 0.1 mM sparsomycin were added as indicated to the complete system described in methods at zero time (first incubation) or after 40 min (second incubation). Experiments were performed as in fig. 2, but in 2 steps: first incubation was for 40 min and the second for 20 min. The exchange of radioactivity was expressed in percentage of the total radioactivity present in the assay. The exchange at 0 min was 3.5%.

Table 2
The effect of spermidine on the exchange of subunits during and after protein synthesis.

Additions during first incubation	Additions during second incubation	Exchange (%)
^{32}P-30 S	–	21.9
^{32}P-30 S + spermidine	–	22.5
–	^{32}P-30 S	22.8
Spermidine	^{32}P-30 S	10.3

Radioactive 30 S subunits and/or 1 mM spermidine were added as indicated. All other conditions as in table 1.

and S_{100} supernatant fluid, both purified using conditions such that the initial reaction mixtures were free of ribosomal subparticles unless they were added.

In a series of experiments we have incubated the system for polypeptide synthesis in the presence of ^{32}P-labeled 30 S particles. At different moments during the reaction, samples were submitted to sucrose gradient centrifugation. Fig. 2 shows the ribosomal profiles at various times during protein synthesis. When the translation proceeded the amount of polyribosomes decreased gradually with a concomitant increase of the 70 S particles.

The resolution of the patterns was fairly good, without any overlapping of the peaks. Therefore the radioactivity which sedimented with the monomer gave a correct measure of the exchange, which did not depend on the exact values of sedimentation coefficients.

The absence of radioactivity in the polysomes region indicates that no reinitiation of polypeptide synthesis occurred under our conditions. Furthermore ^{32}P-labeled 30 S subunits neither exchanged with polysomes nor reattached to them.

The exchange of ribosomal subparticles depends on protein synthesis. When sparsomycin was added at the beginning of the reaction the exchange was completely blocked as a consequence of the inhibition of polypeptide elongation (table 1). This conclusion was confirmed by the fact that the antibiotic *per se* did not affect the exchange, as it could be demonstrated when radioactive subunits and sparsomycin were added after the completion of translation.

Table 2 shows that spermidine did not modify the exchange of subparticles which takes place during protein synthesis, as should be expected if subunits were liberated from mRNA. On the other hand the "post-termination" exchange was clearly reduced by the polyamine, suggesting that under these conditions the 70 S particles were already formed and stabilized by the spermidine.

Our results indicate that in a cell-free system the 30 S and 50 S subunits are released from polysomes simultaneously with the completion of polypeptide chains. The subparticles can then reassociate and reach an equilibrium strongly shifted towards 70 S monomers. This association could be spontaneous or caused by a new factor recently found in our laboratory [10].

Further studies on the different steps of the ribosome cycle which presumably occur between polypeptide synthesis termination and reinitiation of a new round of translation will be published elsewhere.

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