Short Communication

Transitory Ambiguity in Polyuridylic Acid Translation with *Escherichia coli* Ribosomes

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Previous publications (Perzyński, Chomeczyński & Szafrąński, 1969; Chomeczyński, Perzyński & Szafrąński, 1969) from this laboratory have shown that washing of *Bacillus stearothermophilus* ribosomes with 0.5 M-NH₄Cl, dissociation into subunits and purification in 0.2 M-sucrose leads to a several-fold increase in ambiguity during poly(U) translation. This ambiguity can be eliminated by addition of ribosome-free *Escherichia coli* supernatant to *B. stearothermophilus* ribosomes.

In the present study we have demonstrated that properly prepared *E. coli* ribosomes can also exhibit high ambiguity in poly(U) translation and that this effect disappears during storage of the ribosomes.

Materials and methods. *E. coli* strain B was grown as described by Perzyński & Szafrąński (1967) and harvested by centrifugation in the late exponential phase. The cells were disrupted in the Eaton (1962) press in 2 vol. of TNM buffer [10 mM-tris-HCl (pH 7.4)–14 mM-magnesium acetate–70 mM-NH₄Cl–6 mM-2-mercaptoethanol], digested with deoxyribonuclease for 20 min at room temperature and centrifuged at 30 000g for 30 min (S-30 fraction). The ribosomes and S-122 supernatant were isolated from the S-30 fraction as described by Perzyński *et al.* (1969). Part of the ribosomes used for experiments were suspended in TNM buffer, another part in buffer containing 1 M-NH₄Cl [10 mM-tris-HCl (pH 8.1)–10 mM-magnesium acetate–1 M-NH₄Cl].

The tRNA was isolated by the method of Zubay (1962) and acylated as described by Chomeczyński *et al.* (1969).

The binding of aminoacyl-tRNA to ribosomes was measured by the method of Nirenberg & Leder (1964).

The materials were purchased from the following sources: L-[U-¹⁴C]leucine, L-[U-¹⁴C]phenylalanine and L-[G-³H]phenylalanine from The Radiochemical Centre, Amersham, Bucks., U.K.; poly(U) (mol. wt. 64 000) from Calbiochem, Los Angeles, Calif., U.S.A.; deoxyribonuclease (ribonuclease-free) from Worthington Biochemical Corp., Freehold, N.J., U.S.A.

Results and discussion. In the present study we

examined the effect of storing ribosomes in buffer containing 1 M-NH₄Cl on the ambiguity of poly(U) translation. The ambiguity is expressed as the percentage of incorporation of leucine compared with that of phenylalanine in the presence of poly(U). The technique of double-labelling with [¹⁴C]leucine and [³H]phenylalanine was used in the experiments. Initially we examined the ambiguity of translation with ribosomes suspended in TNM buffer. Immediately after isolation of ribosomes this amounted to 4% and did not change with ribosomes stored at 2°C for 9 days. Ribosomes suspended in buffer containing 1 M-NH₄Cl exhibited pronounced changes in ambiguity of poly(U) translation depending on the duration of their storage at 2°C. Fig. 1 illustrates the incorporation of phenylalanine and leucine as well as the ambiguity of translation with ribosomes withdrawn after various periods of storage in buffer containing 1 M-NH₄Cl. These ribosomes were transferred to incorporation medium without sedimentation.

After 30 min of storage in buffer containing 1 M-NH₄Cl, ribosomes already show ambiguity of translation, indicating that the process induced by high salt concentration is a rapid one. The ambiguity of translation of up to 46% persists for about 48 h, decreases to 9% in 3–4 days and remains at this value for several more days. The restoration of fidelity in poly(U) translation is the consequence of a decrease in leucine incorporation and an increase in that of phenylalanine (see Fig. 1).

Thus intact ribosomes that normally translate poly(U) with high fidelity behave biphasically when stored in high salt concentration. Initially they gain the ability for ambiguous translation, but after several days this property disappears. The same results were obtained with ribosomes suspended in buffer containing 1 M-NH₄Cl, sedimented after various periods of storage and used to assay poly(U)-directed incorporation of [³H]phenylalanine and [¹⁴C]leucine.

Since ambiguity in the protein-synthesizing system should depend on codon–anticodon interaction, we investigated whether the poly(U)-directed interaction between [¹⁴C]phenylalanyl-tRNA or [¹⁴C]leucyl-tRNA with ribosomes would confirm our results. It was established that with

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Fig. 1. Changes in ambiguity of translation and protein-synthetic activity with *E. coli* ribosomes stored in buffer containing 1 M-NH₄Cl. The ribosomes were prepared from *E. coli* B cells harvested at the late exponential phase. Samples (10 μ l) of ribosomes suspended in buffer containing 1 M-NH₄Cl (300 *E*₂₆₀ units/ml) were withdrawn at various time-intervals and transferred to the incubation mixture for [³H]phenylalanine and [¹⁴C]leucine incorporation. The incubation mixture contained in 0.25 ml: ribosomes (3 *E*₂₆₀ units); S-122 supernatant fraction (0.4 mg of protein); tris-HCl buffer, pH 7.4 (80 mM); NH₄Cl (70 mM); magnesium acetate (14 mM); ATP (2 mM); GTP (0.5 mM); creatine phosphate (10 mM); creatine kinase (0.012 mg); 2-mercaptoethanol (6 mM); tRNA from *E. coli* B (0.1 mg); poly(U) (0.04 mg); L-[³H]phenylalanine (66.5 Ci/mol; 0.5 mM); L-[¹⁴C]leucine (19.6 Ci/mol; 0.5 mM); mixture of 18 unlabelled amino acids (0.5 mM each). In each experiment a mixture containing no poly(U) was used as a reference sample. After 40 min incubation at 37°C the reaction was stopped by the addition of 10% (w/v) trichloroacetic acid and precipitates were washed as described by Chomczyński *et al.* (1969). Radioactivity of precipitates (³H and ¹⁴C) was measured by a double-labelling technique (De Wachter & Fiers, 1967) with a Packard Tri-Carb scintillation spectrometer. Ambiguity (Δ) is expressed as the percentage of [¹⁴C]leucine incorporation compared with [³H]phenylalanine net incorporation. ●, [³H]Phenylalanine incorporation; ○, [¹⁴C]leucine incorporation.

ribosomes suspended in TNM buffer and transferred to the incubation mixture with 20 mM-Mg²⁺ poly(U) stimulated the binding of phenylalanyl-tRNA whereas no binding of leucyl-tRNA took place. On the other hand, with ribosomes stored in buffer containing 1 M-NH₄Cl poly(U) promoted, under the same conditions, the binding of leucyl-tRNA. The rate of leucyl-tRNA binding to poly(U)-ribosome complex was twice as much during the initial phase of ribosome storage as subsequently.

The results on the binding of phenylalanyl-tRNA and leucyl-tRNA to the poly(U)-ribosome complex confirm in general the changes in ambiguity of translation shown by the studies on the incorporation of leucine and phenylalanine. This suggests that the process of ambiguous translation is related to the codon-anticodon interaction. Studies in this laboratory on the ribosomal proteins and on the degradation of nucleic acids during the storage of ribosomes suggest that differences in fidelity of poly(U) translation, described here, are most probably due to changes in ribosome structure

caused by the 1 M-NH₄Cl treatment. The increase in ambiguity during poly(U) translation has also been observed with rabbit reticulocyte ribosomes exposed to salt (Lamfrom & Grunberg-Manago, 1967). It appears that the phenomena observed are of a more general character and indicate that correct translation of the genetic code is related to ribosomal structure.

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