Short Communications

Fidelity of Polyuridylic Acid Translation in Mixed Systems derived from *Bacillus stearothermophilus* and *Escherichia coli*

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It has been shown (Matthaei, Jones, Martin & Nirenberg, 1962; Bretscher & Grunberg-Manago, 1962) that poly U† can stimulate the incorporation not only of phenylalanine but also of leucine in the *Escherichia coli* cell-free system. This phenomenon has also been observed with the 30000g supernatant from *Bacillus stearothermophilus* (Friedman & Weinstein, 1966). Several factors such as Mg^{2+} and temperature (Szer & Ochoa, 1964), antibiotics (Davies, Gilbert & Gorini, 1964), polyamines (Friedman & Weinstein, 1964) and pH (Grunberg-Manago & Dondon, 1965) are known to influence the fidelity of translation of synthetic polynucleotides in different systems. Since the *E. coli* cell-free system translates poly U with much higher fidelity than that from *B. stearothermophilus*, experiments were performed to study the relative contributions of ribosomes and supernatant in determining the accuracy of translation in mixed systems.

Materials. *B. stearothermophilus* (obtained from the Department of Microbiology, University of Warsaw) was grown at 60° in vigorously agitated medium containing 5g. of yeast extract, 10g. of Proteobak, 5g. of glucose and 5g. of NaCl in 1l.; the pH of medium was 7.2. Cells were harvested in the exponential phase (E_{660} 0.5), washed twice with standard buffer [10mM-tris (pH 7.8)–14mM-magnesium acetate–60mM-KCl–6mM- β -mercaptoethanol] and stored at –40°.

E. coli B was grown and harvested as described by Perzyński & Szafrński (1967).

Cells were disrupted in the Eaton (1962) press, digested with deoxyribonuclease and centrifuged at 30000g for 30 min. (S-30 fraction). The S-30 fraction from *B. stearothermophilus* was preincubated for 10 min. at 60°; the corresponding fraction from *E. coli* for 40 min. at 37° as described by Nirenberg & Matthaei (1961). The S-30 supernatants were centrifuged at 122000g for 2.5 hr., ribosomes were collected and the upper two-thirds

of the resulting supernatants were recentrifuged under the same conditions, dialysed overnight against standard buffer and stored at –40°. Ribosomes were suspended in standard buffer at a concentration of about 300 E_{260} units/ml. ($E_{1\text{cm}}^{1\%}$ 150 at 260 nm.) and used for experiments. The tRNA fractions from *E. coli* and *B. stearothermophilus* were prepared by the method of Zubay (1962).

Chemicals were purchased from the following sources: yeast extract from Difco Laboratories, Detroit, Mich., U.S.A.; Proteobak from Bacutil, Warsaw, Poland; L-[U- ^{14}C]phenylalanine, L-[U- ^{14}C]leucine, L-[4,5- ^3H]leucine from The Radiochemical Centre, Amersham, Bucks.; poly U from Miles Chemical Co., Elkhart, Ind., U.S.A.; deoxyribonuclease (ribonuclease-free) from Worthington Biochemical Corp., Freehold, N.J., U.S.A.

Results and discussion. Initially we examined the effect of purification of ribosomes in 0.5M-NH₄Cl and in 0.2M-sucrose and of their dissociation on the fidelity of poly U translation. *B. stearothermophilus* ribosomes were used and the coding properties of poly U were measured in double-labelling experiments by incorporation of [^{14}C]phenylalanine and [^3H]leucine. The conditions of these experiments were the same as described in Table 1. In some cases [^{14}C]phenylalanine and [^{14}C]leucine were used for incorporation and results obtained were essentially the same as in the double-labelling experiments. When 0.5M-NH₄Cl in the standard buffer was used for purification of ribosomes, ambiguity of poly U translation expressed as the leucine/phenylalanine net incorporation ratio was similar to that for the S-30 fraction and amounted to 6%. When ribosomes were washed with 0.5M-NH₄Cl and purified in 0.2M-sucrose in standard buffer, the leucine/phenylalanine incorporation ratio increased to 11%. With ribosomes washed in 0.5M-NH₄Cl and dissociated in low Mg^{2+} concentration into subunits (Pestka & Nirenberg, 1966) the ambiguity increased to 37%. This value rose to 53% when ribosomes were washed in NH₄Cl, dissociated and additionally purified by centrifugation in 0.2M-sucrose in standard buffer with low Mg^{2+} concentration. Addition of the wash from

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† Abbreviations: poly U, polyuridylic acid; tRNA, transfer or 'soluble' RNA.

Table 1. *Polu U translation in experiments with mixed E. coli and B. stearothermophilus systems*

The incubation mixture contained in 0.25 ml.: ribosomes (0.1 mg. of protein); 122000g supernatant fraction (0.4 mg. of protein); tris-HCl buffer, pH 7.8 (50 mM); NH₄Cl (70 mM); magnesium acetate (14 mM); ATP (2 mM); GTP (0.5 mM); phosphocreatine (10 mM); creatine kinase (0.012 mg.); β -mercaptoethanol (6 mM); tRNA from *E. coli* or *B. stearothermophilus* (0.1 mg.); poly U (0.025 mg.); L-[¹⁴C]phenylalanine (50 mM, specific radioactivity 21 c/mole); L-[³H]leucine (50 mM, specific radioactivity 200 c/mole); mixture of 18 unlabelled amino acids (50 mM each). Samples were incubated at 37° for 40 min. Duplicate assays, both with and without the polynucleotide, were run in all cases. Incorporation was stopped by the addition of 5% (w/v) trichloroacetic acid containing casein hydrolysate (2%, w/v) and washed by the procedure of Barker & Reiber (1967). Radioactivity of precipitates was measured with a Packard Tri-Carb scintillation spectrometer. ¹⁴C and ³H were determined simultaneously with 26% and 28% efficiencies respectively. Endogenous incorporation of phenylalanine amounted to 50–100 pmoles/mg. of ribosomal protein, and that of leucine to about 50 pmoles/mg. The results given in the table represent net incorporation of labelled amino acids.

Source of ribosomes	Supernatant free of ribosomes	Incorporation of amino acids into trichloroacetic acid precipitate (pmoles/mg. of ribosomal protein)		Leucine/phenylalanine incorporation ratio %
		[¹⁴ C]Phenylalanine	[³ H]Leucine	
<i>B. stearothermophilus</i>	<i>B. stearothermophilus</i>	426	224	53
<i>B. stearothermophilus</i>	<i>E. coli</i>	1910	20	1
<i>E. coli</i>	<i>E. coli</i>	6103	241	4
<i>E. coli</i>	<i>B. stearothermophilus</i>	5450	420	8

dissociated *B. stearothermophilus* ribosomes to a homologous system diminished the ambiguity of poly U translation to 20%. The results of these experiments showed that the mode of preparation of *B. stearothermophilus* ribosomes, similarly to reticulocyte ribosomes (Lamfrom & Grunberg-Manago, 1967), plays an important role in the accuracy of poly U translation.

In further experiments we examined poly U translation in mixed systems derived from *B. stearothermophilus* and *E. coli*. *E. coli* ribosomes were prepared by purification in NH₄Cl, dissociated into subunits and washed in sucrose as described above for *B. stearothermophilus* ribosomes. The results of these experiments are shown in Table 1. When a homologous system from *B. stearothermophilus* was used the ambiguity of poly U translation was 53%. The replacement of *B. stearothermophilus* supernatant by *E. coli* ribosome-free supernatant abolished the ambiguity almost completely. In our experiments the *E. coli* cell-free system showed 4% ambiguity. This value was doubled when *E. coli* supernatant was replaced by *B. stearothermophilus* supernatant. All experiments were performed with addition of *E. coli* tRNA. When *B. stearothermophilus* ribosomes and homologous or *E. coli* supernatant were used, tRNA from *B. stearothermophilus* was also added. The results obtained with tRNA fractions derived from both sources were identical.

In some experiments *Pseudomonas fluorescens* P.C.M. 500, which translates poly U with a fidelity similar to that of *E. coli*, was used. Addition of

supernatant from this bacterium to *B. stearothermophilus* ribosomes restored the accuracy of poly U translation similarly to *E. coli* supernatant. This suggests that the above phenomenon may be specific for supernatants derived from systems translating poly U with great fidelity.

In conclusion, the method of preparation of *B. stearothermophilus* ribosomes plays an important role in the fidelity of poly U translation. The highest ambiguity was observed when ribosomes were dissociated into subunits and purified in sucrose. This indicates that, during preparation, ribosomes may lose substances that are involved in the accuracy of translation. Addition of the wash from *B. stearothermophilus* ribosomes to a homologous system restored to a considerable extent the fidelity of poly U translation. Almost complete restoration was obtained when *B. stearothermophilus* supernatant was replaced by *E. coli* or *Ps. fluorescens* dialysed supernatant. This phenomenon does not depend on the biological source of tRNA used in our experiments.

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