Short Communications

The Effect of Soluble Ribonucleic Acid on the Binding of Rapidly ^{14}C -Labelled Ribonucleic Acid to Ribosomes

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Studies on the mechanism of the reaction between s-RNA* and the template-ribosomes complex are usually carried out with synthetic polynucleotides. Nirenberg & Leder (1964) and Kurland (1966) showed that the *Escherichia coli* ribosomes in the presence of polyuridylic acid specifically bind phenylalanyl-s-RNA or its deacylated form. Hatfield (1965) found that phenylalanyl-s-RNA stimulates the binding of trinucleotides, UUC and UUU to the ribosomes. On the other hand, Moore (1966) found no effect of s-RNA on the binding of polyuridylic acid by ribosomes, even in the presence of a 30-fold molar excess of s-RNA over the ribosomes.

Our experiments deal with the effect of s-RNA on the binding of rapidly ^{14}C -labelled RNA to *E. coli* ribosomes.

E. coli B was grown at 32° in a medium containing 5 g. of yeast extract (Difco), 5 g. of casein hydrolysate ('Casamino acids'; Difco), 21.8 g. of K_2HPO_4 , 17 g. of KH_2PO_4 , 0.12 g. of MgSO_4 and 5 g. of glucose in 1 l. Cells were harvested in the exponential phase, washed twice with standard buffer [10 mM-tris (pH 7.8)–14 mM-magnesium acetate–60 mM-KCl–6 mM-mercaptoethanol; Nirenberg & Matthaei, 1961] and stored at –60°.

Ribosomes were obtained from *E. coli* cells disrupted in the Eaton (1962) press. The homogenate was digested with the deoxyribonuclease (ribonuclease-free; Worthington Biochemical Corp., Freehold, N.J., U.S.A.) and centrifuged twice at 30 000 g for 30 min. (S–30 fraction).

The S–30 fraction was preincubated for 90 min. at 37° and the ribosomes were obtained by the method of Nirenberg & Matthaei (1961). The ribosomal pellet was suspended in standard buffer and stored at –60°. The concentration of ribosomes was estimated spectrophotometrically at 260 m μ ($E_{260}^{1\%}$, 150). Their molecular weight was assumed to be 2.6×10^6 (Petermann, 1964).

s-RNA was isolated from the total RNA obtained from *E. coli* cells by the sodium dodecyl sulphate-phenol method (Scherrer & Darnell, 1962). The crude RNA was digested with 2 μg . of deoxyribo-

nuclease (ribonuclease-free)/ml. and fractionated with 1.5 M-NaCl. s-RNA was deacylated in tris buffer, pH 8.8, at 37° for 60 min. and purified on Sephadex G-100 (Schleich & Goldstein, 1966). The s-RNA fraction was dialysed against standard buffer and stored at –20°.

The molecular weight of s-RNA was taken as 2.56×10^4 (Lindahl, Adams & Fresco, 1966).

The rapidly ^{14}C -labelled RNA was obtained from *E. coli* cultivated on glucose-casein-hydrolysate medium (Baldwin & Shooter 1963). In the exponential phase of growth, [^{14}C]adenine (The Radiochemical Centre, Amersham, Bucks.), of specific activity 28.9 mc/m-mole, was added to give a final concentration of 0.12 mM. After 4 min. unlabelled adenine was added to give a final concentration of 4 mM, and the culture was rapidly chilled and centrifuged. RNA was obtained by the sodium dodecyl sulphate-phenol method. The crude ^{14}C -labelled RNA was filtered through Sephadex G-100 in 0.1 M-NaCl: 90% of the total radioactivity applied to the column emerged in the peak of high-molecular-weight RNA. The specific activity of this RNA measured in the liquid-scintillation counter was 955 counts/min./ μg . of RNA.

Interaction between rapidly ^{14}C -labelled RNA, ribosomes and s-RNA. At the beginning the reaction between ^{14}C -labelled RNA and ribosomes was investigated. A 5.6 μg . sample of ^{14}C -labelled RNA (measured at 260 m μ) and various quantities of ribosomes were incubated in 0.3 ml. of standard buffer at 23° for 6 min. The samples were then diluted with 3 ml. of the same buffer, filtered immediately on a Millipore HA 0.45 μ filter (Millipore Filter Corp., Bedford, Mass., U.S.A.) and washed with 15 ml. of standard buffer (Nirenberg & Leder, 1964). The filters were dried and counted in a scintillation counter. The highest rate of binding of ^{14}C -labelled RNA to ribosomes was observed with 220 μg . of ribosomes, and 25% of the ^{14}C -labelled RNA was attached to this amount of ribosomes. Since the ribosomal RNA present in the preparation of ^{14}C -labelled RNA does not react with ribosomes (Ishihama, Mizuno, Takai, Otaka &

* Abbreviation: s-RNA, 'soluble' RNA.

Fig. 1. Effect of s-RNA on the interaction of rapidly ^{14}C -labelled RNA with ribosomes. The mixture contained the components added in the following sequence: standard buffer [10 mM-tris (pH 7.8)–14 mM-magnesium acetate–60 mM-KCl–6 mM-mercaptoethanol], 5.6 μg . of ^{14}C -labelled RNA, various amounts of s-RNA, 220 μg . of ribosomes; the final volume was 0.3 ml. The samples were incubated at 23°, diluted, filtered on Millipore filters and counted (see the text).

Osawa, 1962), it may be supposed that the radioactivity bound to ribosomes corresponds to messenger RNA. In some experiments the amount of the rapidly ^{14}C -labelled RNA attached to ribosomes was estimated by centrifugation in a discontinuous sucrose gradient (Wettstein, Staehelin & Noll, 1963). The results obtained with the latter method were consistent with those found by the Millipore-filter technique.

The effect of s-RNA on the binding of ^{14}C -labelled RNA to ribosomes was also studied with both the methods described above and similar results were obtained. The amount of ^{14}C -labelled RNA bound to ribosomes decreases by up to 53% with the increase in the concentration of s-RNA (Fig. 1). The inhibitory effect of s-RNA is observed when its molar concentration is about fivefold that of the ribosomes (12 μg . of s-RNA/220 μg . of ribosomes). Similar results were observed at 15°, 23°, 36° and 45°. This effect of s-RNA on the binding of the template to ribosomes is not due to the dilution of ^{14}C -labelled RNA with unlabelled RNA, since s-RNA was free of high-molecular-weight RNA. On the other hand, it is not a result of the action of nucleolytic enzymes, since ^{14}C -labelled RNA is not decomposed in the presence of s-RNA. Thus it might be supposed that the binding of messenger RNA to the ribosomes depends on the transfer RNA, although the effect of some contamination occurring in s-RNA cannot be excluded.

Studies on the interaction between s-RNA, ^{14}C -labelled RNA and ribosomes showed that the

preincubation of the components and the sequence of their addition affect the rate of interaction. The amount of ^{14}C -labelled RNA bound to ribosomes in the absence of s-RNA was considered as 100%. When s-RNA was added to the ribosomes preincubated with ^{14}C -labelled RNA, about 70% of the radioactivity remained bound to ribosomes. However, when the ribosomes were added to ^{14}C -labelled RNA, preincubated with s-RNA, only 47% of the radioactivity was attached to ribosomes. The preincubation of ribosomes with s-RNA and the subsequent addition of ^{14}C -labelled RNA yielded the same results as in the latter case. Chlortetracycline, which blocks the binding of s-RNA to ribosomes by 50% (Hierowski, 1965), did not exert any influence on the interaction in the experiments described above. This suggests that the inhibitory effect of s-RNA on the binding of template to ribosomes is not due to the attachment of s-RNA to the ribosomes. It might be suggested that s-RNA, bound to messenger RNA by anticodons and codons, decreased the ability of binding of the template to ribosomes.

It seems that the phenomenon described above may be responsible for the previously observed inhibitory effect of s-RNA on the incorporation of amino acids into proteins in cell-free systems (Tanaka & Shimura, 1965; Aaronson, Korner & Munro 1966).

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