SPECIFIC INTERACTION OF SOLUBLE RNA WITH POLYRIBONUCLEIC ACID INDUCED POLYSOMES*

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Despite the recent remarkable progress made on the elucidation of the genetic code (Nirenberg et al., 1963; Ochoa, 1963) the exact biochemical mechanism by which a messenger RNA directs specific protein synthesis has remained obscure. It has been reported that synthetic messenger RNA such as polyuridylic acid (poly-U) causes aggregation of ribosomes resulting in the formation of so-called polysomes (Barondes and Nirenberg, 1962; Spyrides and Lipmann, 1962). In this preliminary communication we report that specific S-RNA attaches to the polysomes produced by a synthetic polyribonucleic acid. In other words, phenylalanine S-RNA attaches to the polysomes produced by poly-U and lysine S-RNA attaches to those produced by polyadenylic acid (poly-A). The interaction is specific in that only the particular amino acid S-RNA coded by the polynucleotide interacts with the polysomes. Using this principle it has been possible to obtain phenylalanine S-RNA which is virtually free of other amino acid S-RNA so far tested. Detailed account of the present work will be published elsewhere.

E. coli B was grown in a minimal salts medium with glycerol as a carbon source. Cells were harvested at early logarithmic phase and the "incubated S-30"

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(is-30) was prepared as described by Nirenberg and Matthaei (1961). In some cases concentrated is-30 (conc. is-30) was prepared by centrifugation of is-30 at 105,000 g for 2 hrs and resuspending the ribosomes in the 1/10 of the resulting supernatant fluid. The concentration of the ribosome in conc. is-30 is therefore ten times higher than that of is-30. E.coli S-RNA was prepared as described by Ofengand et al. (1961). In order to prepare amino acyl S-RNA synthetase (charging enzyme) from E.coli, the extracts were prepared as described previously (Kaji et al., 1963) and the supernatant solution obtained after centrifugation of protamine precipitate was used as a starting material. From this supernatant fluid a protein fraction precipitated by ammonium sulfate (between 30 and 46% saturation) was collected and dissolved in minimum volume of buffer containing 0.01 M tris HCl pH 7.5, 0.01 M magnesium acetate and 0.01 M β-mercaptoethanol. The enzyme was dialyzed against the same buffer for 12 hrs at 4°C and stored at -20°C. The charging enzyme prepared in this fashion was free from S-RNA or the soluble amino acid incorporating activity (Kaji et al., 1963). To measure the amount of S-RNA specific to certain amino acid, each fraction from density gradient centrifugation was incubated with the charging enzyme in the presence of the C^{14}-amino acid, energy generating system and metal ions. The radioactive amino acyl S-RNA formed is soluble in hot TCA (trichloroacetic acid) and insoluble in cold TCA. Radioactivity incorporated into the protein fraction is insoluble in both hot and cold TCA. It is therefore possible to measure the amount of an individual amino acyl S-RNA by subtracting the radioactivity insoluble in hot TCA from that insoluble in cold TCA. Subtraction of hot TCA insoluble radioactivity is necessary because part of the incorporated radioactivity which is insoluble in cold TCA is due to the radioactive protein formed. In some cases the transfer of amino acid from S-RNA to protein was prevented by the addition of puromycin into the S-RNA assay mixture. The radioactive amino acyl S-RNA was in some cases determined by measuring the radioactivity which was insoluble in cold TCA but was rendered soluble by treatment with pancreatic RNase.

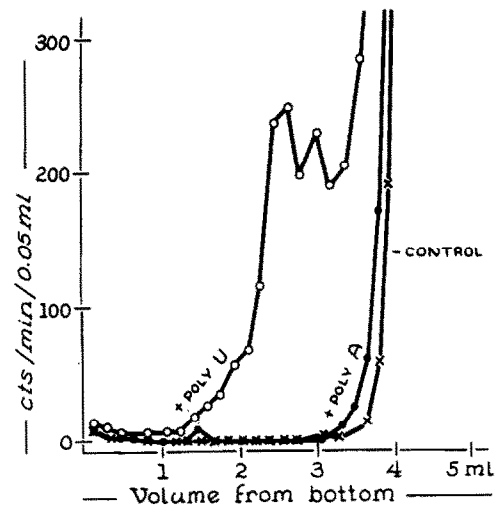

Figure 1. Attachment of Amino Acyl S-RNA to Polysomes.

Reaction mixture contained in a final volume of 0.55 ml 50 μ moles of tris (pH 7.8), 5.6 μmoles of magnesium acetate, 3.0 μmoles of KCl, 2.0 μmoles of β-mercaptoethanol, 2.0 μ moles of ATP (K salt), 0.2 μmole of GTP, 10 μg of pyruvate kinase (Böhringer), 4 μmoles of phosphoenol pyruvate, 0.5 mg of S-RNA, 22 mg of sucrose, approximately 340,000 cpm of C^{14}-phenylalanine (200 μc/μmole, counting efficiency: 1 μc = 10^6 cpm), and 1.8 mg of is-30. Where indicated, 260 μg of poly-U or poly-A (obtained from Miles Laboratory) was added. Of this reaction mixture, 0.3 ml was taken and placed on top of 4.7 ml of a linear sucrose gradient (5% to 20%) containing 0.1 M tris HCl (pH 7.8), 0.01 M magnesium acetate and 0.05 M KCl. The tube was centrifuged for 1 hr at 37,000 rpm in the SW 39 L rotor of the Spinco Model L centrifuge at 2 — 5°C. After centrifugation, two drop fractions were collected in test tubes by puncturing the bottom of the tube. Two aliquots of 0.05 ml were taken from each fraction, one was used for measuring radioactivity insoluble in hot TCA (Mans and Novelli, 1960) and the other was processed in the identical way except for omission of the hot TCA step for the measurement of cold TCA insoluble radioactivity. The difference of counts of these two samples is plotted against the volume collected from the bottom of the centrifuge tube.

In the experiment shown in Fig. 1, is-30 was mixed with C^{14}-phenylalanine, S-RNA, an energy generating system, GTP and poly-U. The mixture was immediately subjected to the sucrose gradient centrifugation. The formation of amino acyl S-RNA is so rapid under the present experimental conditions that the reaction mixture does not require incubation. The distribution of C^{14}-phenylalanyl S-RNA formed in the reaction mixture on the density gradient centrifugation was plotted against the fraction of the total volume measured from the bottom of the tube. It can be seen from this figure that

poly-U shifts the distribution of C^{14}-phenylalanyl S-RNA toward the bottom of the centrifuge tube. In the control experiment where poly-A was added instead of poly-U, there was no shift of phenylalanyl S-RNA distribution. In a separate experiment where C^{14}-lysine, instead of C^{14}-phenylalanine was used, only poly-A but not poly-U shifted the distribution of C^{14}-lysyl S-RNA toward the bottom of the tube.

In order to see if free S-RNA without amino acid would also interact with the polysomes, the experiment shown in Fig.2A was performed. In this experiment, is-30, S-RNA, GTP, energy generating system and poly-U was mixed in the absence of labeled amino acid. After the sucrose density gradient centrifugation of the mixture, the amount of various amino acid S-RNA in each fraction was determined by measuring the incorporation of C^{14}-amino acid into cold TCA insoluble but hot TCA soluble fraction. It is clearly shown in this figure that only the distribution of phenylalanine S-RNA is shifted toward the bottom of the tube by the influence of poly-U. In a similar experiment using poly-A (Fig.2B), the distribution of lysine S-RNA is now shifted toward the bottom of the tube. It is concluded from these results that free S-RNA can interact with polysomes produced by specific polyribonucleotides. It should be pointed out that the fractions toward the bottom contained only the particular amino acid S-RNA corresponding to the code words in the polynucleotide and were virtually free from other amino acid S-RNA. As controls, separate experiments with no added polynucleotides or with added polyribonucleotide lacking the code words for the amino acid were performed. In these experiments, no shift of S-RNA toward the bottom of the centrifuge tube was observed indicating that the effect of synthetic polynucleotide is specific to the amino acid S-RNA coded for in the polynucleotide.

Data shown in Fig.3 are the results of a similar experiment carried out using conc. is-30. It should be noted that under these conditions, the majority of phenylalanine S-RNA is now bound to the poly-U induced polysomes. The reason is that the ratio of polysomes to S-RNA in this reaction mixture is much higher than the one in the

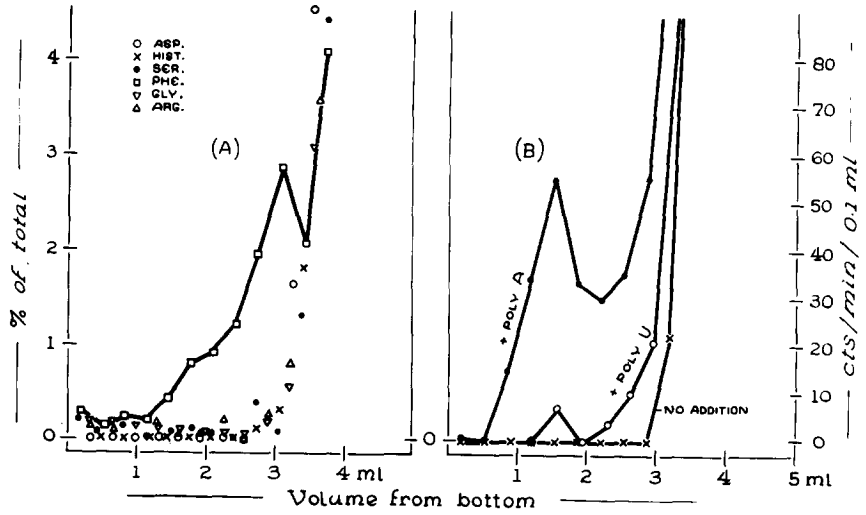

Figure 2. Specific Attachment of S-RNA to Polysomes

A) The reaction mixture was identical to that of Fig. 1 except for the following: It contained 3.6 mg of is-30, 1 mg of poly-U, but no labeled amino acid was added. Three identical reaction mixtures were prepared and subjected to the sucrose density centrifugation simultaneously as in Fig. 1. Each fraction was analyzed for various amino acid S-RNA. Fractions from tube 1 were used for analysis of phenylalanine, and arginine S-RNA, tube 2 was used for aspartic acid and histidine S-RNA, and tube 3 was used for glycine and serine S-RNA. The reaction mixture for measuring S-RNA contained the following in μmole/0.5 ml: 50 tris, pH 7.5; 1 $MnCl_2$; 2.4 magnesium acetate; 2 β-mercaptoethanol; 2 ATP (K salt); 4 phosphoenolpyruvate, K salt; 50 μg phosphoenolpyruvate kinase (crystalline); 0.1 ml of each fraction from the density gradient centrifugation; 0.5 mg of charging enzyme and one kind of C^{14}-amino acid. The amount of labeled amino acid added was as follows in cpm/0.5 ml: 110,000 phenylalanine (200 μc/μmole); 140,000 arginine (250 μc/μmole); 320,000 serine (140 μc/μmole); 240,000 glycine (80 μc/μmole); 100,000 histidine (133 μc/μmole); 240,000 aspartic acid (100 μc/μmole). For the assay of S-RNA, the incubation was carried out for 10 min at 37°C and two 0.1 ml aliquot were taken. One was processed for measuring incorporation into protein fraction (Mans and Novelli, 1960), and the other aliquot was processed in the identical way except for omission of the hot TCA treatment. The difference of these two counts is taken as amino acyl S-RNA. The values are expressed as percentage of each of total individual amino acid S-RNA recovered. Total radioactivity recovered as amino acyl S-RNA are as follows in cpm/0.1 ml reaction mixture for the S-RNA assay: 6,630 phenylalanine; 2,800 arginine; 2,000 serine; 10,000 glycine; 22,000 histidine; 2,400 aspartic acid.

B) The reaction mixture was identical to (A) except in some case 1 mg of poly-A was added instead of poly-U or polynucleotide was omitted as indicated in the figure. After the sucrose density gradient centrifugation as in Fig. 1, each fraction was analyzed for lysine S-RNA. The reaction mixture for S-RNA analysis was identical to those in A except C^{14}-lysine (150,000 cpm/0.5 ml, 250 μc/μmole) was added as a radioactive amino acid. The cold TCA insoluble, RNase sensitive radioactivity/0.1 ml of the assay mixture is plotted against the volume collected from the bottom of the centrifuge tube.

preceding experiment. There was no binding of arginine S-RNA to the poly-U induced polysomes.

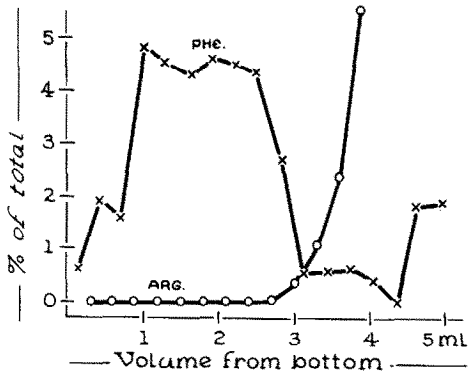

Figure 3. Specific Attachment of Phenylalanine S-RNA to Poly-U Induced Polysomes.

The reaction mixture was identical to that of Fig. 2A except it contained 3 mg of conc. is-30, 260 µg of poly-U. After centrifugation, fractions were analyzed for arginine and phenylalanine S-RNA using the conditions described in Fig. 2A. The reaction mixture for S-RNA assay was identical to that in Fig. 2A except it contained 5×10^{-4} M puromycin, 220,000 cpm of C^{14}-phenylalanine (200 µc/µmole) or 360,000 cpm of C^{14}-arginine (250 µc/µmole), and 0.1 ml of each fraction from the sucrose density gradient centrifugation in final volume of 0.3 ml. The total amino acyl S-RNA recovered was 9,000 cpm of arginyl S-RNA and 4,400 cpm of phenylalanyl S-RNA per 0.1 ml of the assay mixture.

It appears that the present finding has three-fold significance. It indicates that interaction of messenger RNA with S-RNA takes place prior to and independent of the peptide bond synthesis. The indication is that ribosome bound messenger RNA has a much stronger affinity for S-RNA than free messenger RNA, and the S-RNA interacts with messenger RNA only when the latter is bound to ribosomes. Secondly, the principle described here might well be used for the preparation of pure amino acid transfer S-RNA. The observation that the fractions toward the bottom of the centrifuge tube contained phenylalanine S-RNA almost free from other amino acid S-RNA tested (Fig. 2A) strongly suggest the usefulness of this method for preparation of pure individual amino acid S-RNA. Thirdly, the present system might provide a new method

to determine the coding ratio. Since poly-U and the attached S-RNA on the ribosome can be measured accurately, one can measure the coding ratio without introducing the error caused by endogenous degradative enzymes. It has further advantage of avoiding the error caused by repeated functioning of the messenger RNA. Work is in progress to eludicate these points mentioned above.

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