CHARACTERISTICS OF ACTIVATION OF RIBOSOMES FROM EHRLICH ASCITES TUMOUR CELLS WITH POLYURIDYLIC ACID*

S. Pedersen, A.M. White** and T. Hultin

The Wenner-Gren Institute, University of Stockholm, Sweden

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Current evidence indicates that active ribosomes are joined by a strand of "messenger" RNA to form functional aggregates, polysomes. It is generally assumed that the individual particles forming these aggregates attach at one end of the messenger RNA strand and gradually move forward to coincide with the formation of new peptide bonds (Gierer, 1963; Gilbert, 1963; Warner et al., 1963; Wettstein et al., 1963).

The concept of a continual ribosomal displacement coupled to the peptidisation mechanism seems to imply that the attachment of more than one ribosome to a messenger RNA strand, i.e. polysome formation, would take place only under conditions of active protein synthesis. A requirement for nucleoside triphosphates and soluble enzymes would accordingly be expected. In experiments with bacterial ribosomes it has been shown, however, that the presence of the artificial messenger RNA polyuridylic acid (poly-U) gives rise to a spontaneous formation of polysome-like aggregates in which virtually all the activity for phenylalanine incorporation is concentrated (Barondes and Nirenberg, 1962; Spyrides and Lipmann, 1962). In view of the above considerations it seemed of interest to know

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** Present address: Research Division, CIBA Laboratories Ltd., Horsham, Sussex, England.

whether such spontaneous aggregation of ribosomes in the presence of poly-U is a general phenomenon also characteristic of non-bacterial ribosomes.

Experimental

Ehrlich ascites tumour cells (1 volume) were collected in 0.9% NaCl, washed with 0.009 M $MgCl_2$ and carefully homogenised with 2.2 volumes of the same medium in a Dounce homogenisor without disruption of the nuclei. The ionic composition of the suspension was adjusted, by the addition of 0.8 volumes of concentrated medium, to 0.075 M KCl, 0.009 M $MgCl_2$, and 0.035 M Tris buffer (pH 7.8). The homogenate was centrifuged for 7 min at 15,000 x g. Gradient centrifugations were run for 2-2.5 hrs at 53,000 x g (Spinco rotor SW 25) using an exponential sucrose gradient (usually 10-25%) prepared in the same buffered salt solution. Fractions of known volume were collected after puncturing the bottom of the tube with a needle. Cell sap was prepared from the 15,000 x g supernatant by centrifugation for 2 hrs at 105,000 x g.

The 15,000 g supernatants were treated with poly-U (0.5 mg/ml) at 0°C for 5 min. Of the fractions obtained after gradient centrifugation 0.6 ml samples were tested for activity of amino acid incorporation using a system containing 0.22 ml cell sap, 10 µmoles phosphoenol pyruvate, 1 µmole ATP, 0.2 µmoles GTP, 0.015 µg pyruvate kinase and 16.5 mµmoles of ^{14}C-L-phenylalanine (61.9 mC/mmole) or 46.5 mµmoles of ^{14}C-L-leucine (21.5 mC/mmole). Final volumes were 1.0 ml. After 8 min of incubation at 35°C the proteins were precipitated with trichloroacetic acid as described previously (Pedersen and Hultin, 1963).

In experiments involving pretreatment with tritiated poly-U, fractions from the density gradient were diluted with buffered salt solution, layered on 7.5 ml of medium containing 0.44 M sucrose, and centrifuged for 2 hrs at 105,000 g. The radioactivity of the sediments

was determined in a liquid scintillation counter (Packard Tricarb).

Polyuridylic acid was obtained from the Miles Chemical Co., N.J. ^{3}H-Polyuridylic acid was prepared from tritiated UDP by incubation with polynucleotide phosphorylase (Grunberg-Manago, 1956).

Results

The centrifugation pattern of the postmitochondrial fraction of Ehrlich cells showed a large 70-80S peak, preceded by less marked absorption bands which extended to the bottom of the tube where a small pellet always accumulated (Fig. 1).

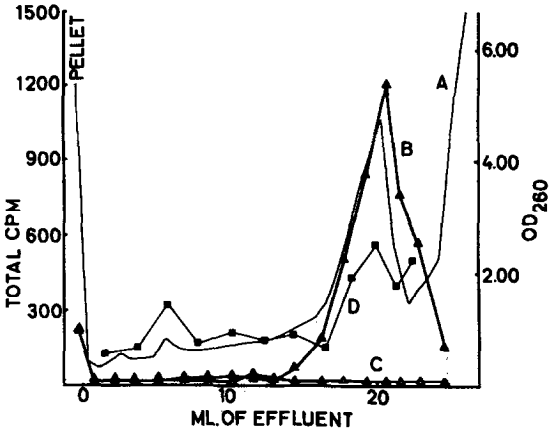

Fig. 1. Distribution of endogenous and poly-U-induced activity of phenylalanine incorporation among density gradient fractions of Ehrlich cell ribosomes. The 15,000 g supernatant was kept for 8 min at 35°C and then incubated for 5 min at 0°C in the presence or absence of poly-U or tritiated poly-U (0.5 mg/ml). Density gradient analyses were run for 2 hrs on 1.5 ml samples. A. Optical density of effluents (mean values). B,C. Phenylalanine incorporation activity of ribosome fractions prepared after preincubation with or without poly-U, respectively. No poly-U was included in the incorporation system. D. Distribution of particle-bound isotope among the ribosome fractions after preincubation with tritiated poly-U. Zero point values refer to particles sedimented during density gradient centrifugation and diluted to the same volumes as the standard fractions.

In several experiments one of these bands was especially prominent. This band sedimented about 3 times faster than the 70-80S particles. The endogenous activity for phenylalanine and leucine incorporation was con-

centrated in the pellet and the rapidly sedimenting bands (Figs. 1,2). Phenylalanine incorporation in the presence of poly-U (0.5 mg/ml) was, however, especially high in the 70-80S fraction.

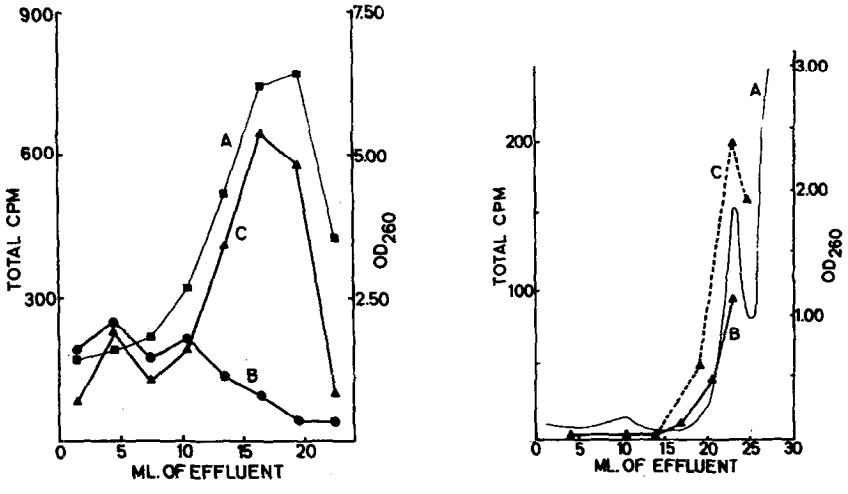

Fig. 2. Distribution pattern of poly-U-induced incorporation activity with respect to phenylalanine and leucine. Experimental details as in Fig. 1, except that density gradient centrifugations were run for 2.5 hours. A. Optical density of effluent. B, C. Activity of ribosome fractions, prepared after poly-U preincubation, for the incorporation of leucine and phenylalanine, respectively.

Fig. 3. Non-aggregation of poly-U-pretreated ribosomes in the course of phenylalanine incorporation. A 15,000 g supernatant (6.0 ml) was preincubated with poly-U for 5 min at 0°C and submitted to gradient density centrifugation in 3 tubes. From the top region of the 70-80S fraction in each tube 2.0 ml were collected. Samples of this suspension were incubated for 2 or 6 min (35°C) in a complete phenylalanine incorporating system and then subjected to a second density gradient analysis. Pooled fractions of the effluent were precipitated with trichloracetic acid in the presence of a protein carrier, and the total radioactivities of the purified proteins were determined. A. Mean optical density of effluents from the second density gradients. B, C. Distribution of protein-bound radioactivity after incubating the poly-U-pretreated particles in a phenylalanine incorporation system for 2 and 6 min, respectively.

Particles from the 15,000 x g supernatant fractions which had been pretreated with poly-U retained most of their poly-U-dependent phenyl-

alanine incorporating ability after density gradient centrifugation (Pedersen and Hultin, 1963). This retained activity was almost exclusively concentrated in the fraction of 70-80S particles. In the experiments with tritiated poly-U, however, some of the bound radioactivity was always associated with the heavier particles (Fig. 1). The proportion of labelled poly-U bound to these particles was largely independent of the poly-U concentration during preincubation (0.02-0.5 mg/ml) (Okamoto and Takanami, 1963). Since the heavier particles did not show any poly-U dependent incorporation ability it was concluded that poly-U was bound to these particles essentially in a non-functional manner.

As intimated in the introduction one might expect a formation of polysome-like aggregates in connection with actual phenylalanine incorporation. In the experiments shown in Fig. 3, 70-80S particles, pretreated with poly-U, were isolated by gradient density centrifugation, and then incubated for 2 or 6 min in a complete phenylalanine incorporating system. These suspensions were then analysed for radioactivity distribution by a second density gradient centrifugation. A small, but non-poly U dependent peak was constantly observed in the heavy region of the gradient (von der Decken, 1961). Nevertheless, labelled proteins were in all cases present exclusively in the large 70-80S peak.

Our experiments seem to indicate that Ehrlich cell ribosomes are incapable of forming functional aggregates spontaneously under the influence of poly-U. Thus it appears that such aggregates are not obligatory for phenylalanine incorporation. If active aggregates of a transient nature were formed during incubation these would in all probability have been observed in the short-term experiments shown in Fig. 3. According to current concepts polysomes should be formed, but they may require for their demonstration the presence of free poly-U in the incorporation system or the use of a poly-U of greater chain length during preincubation.

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