DIFFERENCES IN THE RIBOSOMAL PROTEIN OF FREE AND MEMBRANE BOUND POLYSOMES OF CHICK EMBRYO CELLS [1]

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Summary

Two classes of polysomes, free and membrane bound, were isolated from chick embryo tissue cultures. Polysomes of both classes are present in about equal amounts. Polyacrylamide gel electrophoresis revealed at least two differences in the ensemble of ribosomal protein of the two polysome classes.

Introduction

Many animal cells contain at least two classes of ribosomes, free and bound to membranes. Several lines of evidence indicate that the translation of certain messenger RNA (m-RNA) is restricted to one of the two classes of ribosomes (1, 2). Little is known so far about the mechanisms involved in directing m-RNA to a specific site for protein synthesis. A possible mechanism could be a difference in ribosome structure which would restrict the association and translation of a class of m-RNA to one class of ribosomes.

We would like to report at least two differences between the ensemble of proteins isolated from free and membrane bound polysomes (bound polysomes).

Materials and Methods

Tissue cultures: Primary tissue cultures were prepared from twelve day old chick embryos (3). The tissue cultures were grown in Hanks or Eagle's media with 8% calf serum in tightly closed 200 ml bottles at 35°C.

Isolation of Polysomes: Three day old cells were harvested by trypsini-

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zation after washing the monolayers with hypertonic buffer (0.25M KCl, 0.01M $MgCl_2$, 0.01M Tris-HCl pH 7.4) (4). The cells were resuspended in 0.02% lima bean trypsin inhibitor and centrifuged 5 minutes at 755 x g. The cell pellet was resuspended in hypertonic buffer, frozen and thawed once. This method of cell lysis has been worked out previously and was shown to be very efficient for chick embryo cells (5). The cell lysate was centrifuged 2 minutes at 5,900 x g. The free polysomes were isolated from the supernatant and the bound polysomes from the pellet. A small amount of membrane material was removed from the supernatant by centrifugation for 5 minutes at 12,100 x g. This supernatant was treated with Tween 40 (Mann Research Laboratories) and sodium desoxycholate (DOC) at final concentrations of 1% and 0.5% respectively and centrifuged for 5 minutes at 27,000 x g.

The pellet containing the bound polysomes and other rapidly sedimenting cell components was washed once in hypertonic buffer and was then resuspended in hypertonic buffer containing 1% Tween 40 and 0.5% DOC. Nuclei and other nonsolubilized material were removed by centrifugation for 5 minutes at 27,000 x g.

Free and bound polysomes were separated from single ribosomes, ribosomal subunits and soluble proteins on concave exponential (6) 0.5M to 1.5M sucrose gradients prepared in hypertonic buffer. All operations were carried out at 0-4°C.

Extraction and analysis of ribosomal protein: Purified polysomes were pelleted from the sucrose gradient fractions by centrifugation for 2 hours at 269,245 x g. The polysome pellets were resuspended in 0.2 ml of 0.01M phosphate buffer (pH 7.1) containing 2% sodium dodecyl sulfate (SDS), 0.5% urea and 2% 2-mercaptoethanol. The mixture was incubated at 37°C for 1 hour and dialyzed overnight against 0.01M phosphate buffer (pH 7.1) containing 0.5% SDS, 0.5% urea and 2% 2-mercaptoethanol. Gel electrophoresis was performed on 18 cm polyacrylamide gels as described by Stevens et al. (7).

Results

Distribution of polysomes: The sucrose gradient analysis of the cell extracts containing the free polysomes and the detergent released bound polysomes are shown in Fig. 1A and B. Both optical density profiles represent the extracts from the same number of cells, hence by integrating the area under the appropriate part of the profile the distribution between the two extracts of ribosomal subunits, single ribosomes and polysomes can be calculated. These calculations showed that polysomes and large ribosomal subunits are about equally distributed between the two extracts, while single ribosomes and small ribosomal subunits are predominantly present in the extract containing the free polysomes. Direct microscopic inspections were carried out routinely after thawing the cells in order to ascertain that the two extracts do not result from lysis of only half the cells by freezing and thawing and lysis of the

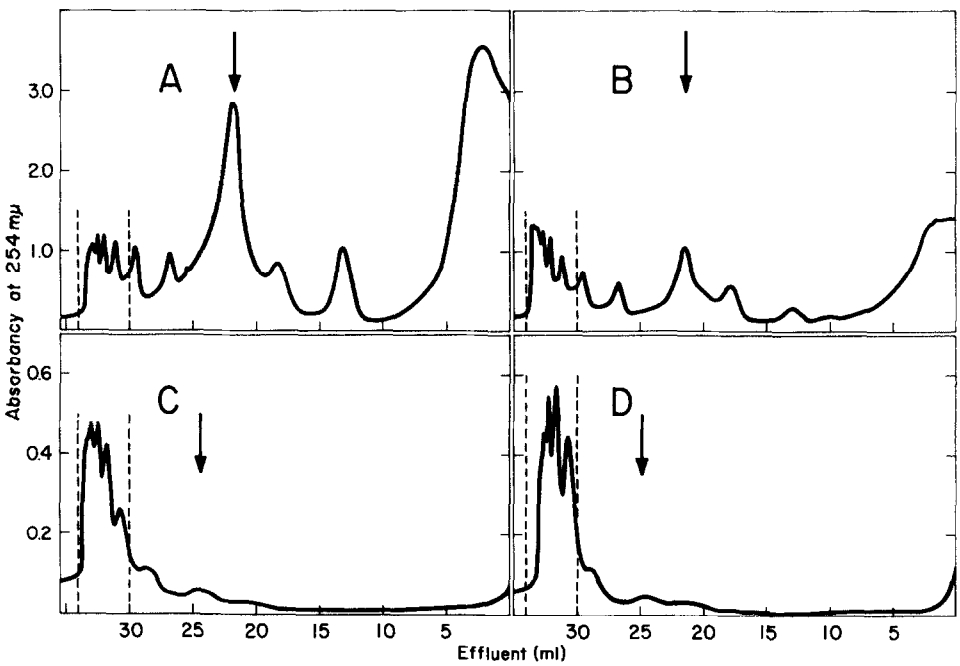

Fig. 1. Distribution of polysomes from three day old tissue cultures. The top of the gradients are to the right, the bottom to the left. The arrow indicates the position of single ribosomes. Centrifugation was for 16 hours at 52,200 x g in an SW 27 rotor in A and B, for 17 hours at 55,100 x g in C and D: (A) extract containing free polysomes (B) extract containing bound polysomes (C) recentrifuged bound polysomes (D) recentrifuged free polysomes.

other half by detergent treatment. These microscopic inspections showed that at least 95% of the cells were broken by freezing and thawing. The polysomes made of four or more ribosomes were pooled as indicated in Fig. 1A and B, dialyzed against hypertonic buffer and further purified on a second sucrose gradient (Fig. 1C and D).

Gel electrophoresis analysis of ribosomal protein: Protein extracted from polysomes purified by two cycles of sucrose gradient centrifugation was separated by polyacrylamide gel electrophoresis for 24 hours as described. The gels were stained overnight with Coomassi Brilliant Blue (8). Photographs of the stained gels (Fig. 2) show that the protein is resolved into several bands. Protein band 1 is very weak and could only be detected in bound polysomes, while protein band 2 is present only in free polysomes.

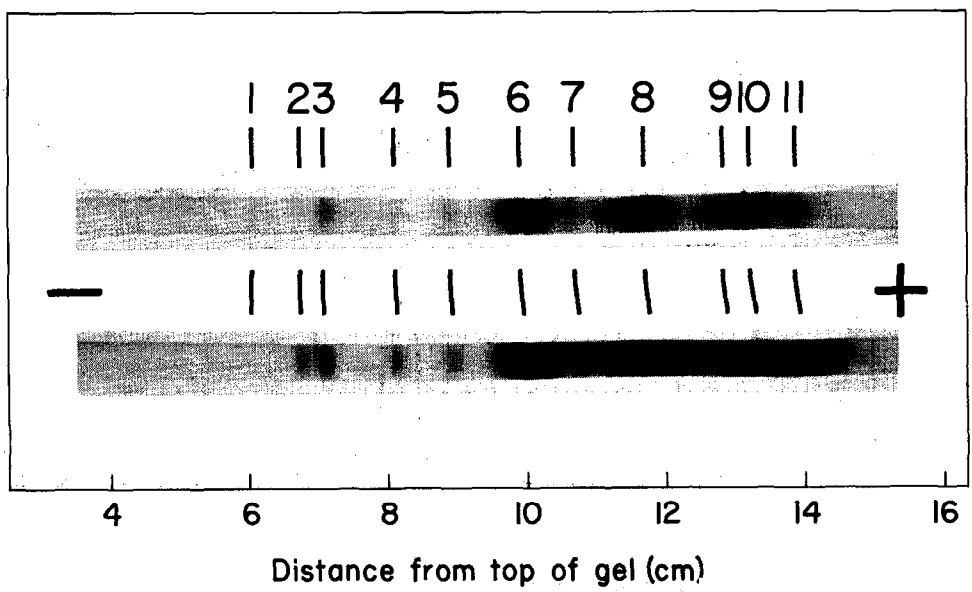

Fig. 2. Polyacrylamide gel electrophoresis of ribosomal protein. 7.5% polyacrylamid gels were prepared, electrophoresed and stained as described in the text. Electrophoresis was from left to right. Top: membrane bound polysomes. Bottom: free polysomes.

Similar results were obtained when protein labeled with ^{3}H or ^{14}C-leucine *in vivo* was analyzed (Fig. 3). Here protein band 1 is easily detectable and is much more pronounced in the bound polysomes. The difference in the relative intensity of band 1 between the stained gels and the labeled gels may be attributable to a low relative affinity of the protein in this band for the stain (9) or a high relative content of leucine. There are not two distinct peaks of radio label in the position of the protein bands 2 and 3 of the free polysomes due to a loss in resolution, because 1.8 mm gel fractions were counted. However, it can be readily seen that the labeled protein band from the free polysomes is shifted toward the position where in stained gels protein band 2 is found. This shift indicates a partial or complete absence of label in bound polysomes in the position of protein band 2 of free polysomes, thus confirming the results obtained with stained gels. The same difference in the

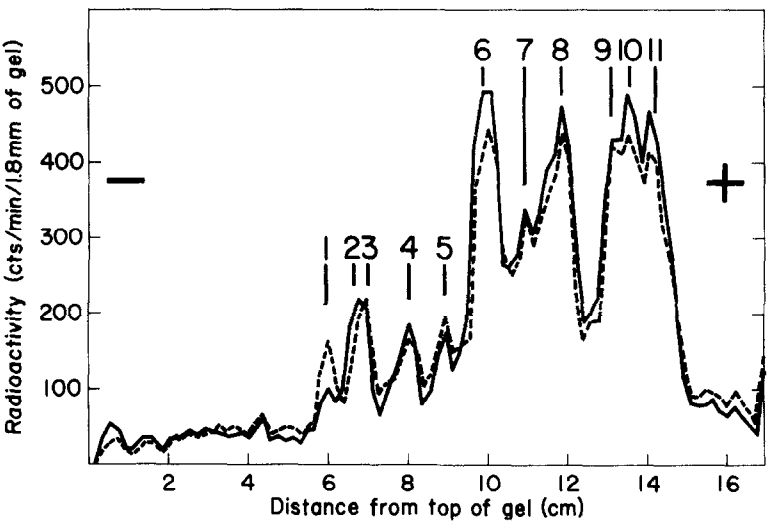

Fig. 3. Polyacrylamide gel electrophoresis of ribosomal protein labeled *in vivo*. The fluid of tissue cultures grown for one day in Hank's media, was replaced with the same media containing 1/20 of the normal amount of lactalbumin and 38 µc of ^{3}H-leucine or 7.5 µc of ^{14}C-leucine per bottle. 44 hours later the radio labeled medium was removed and the cells were incubated for an additional five hours in unlabeled Hank's media. Ribosomal protein from purified polysomes was analyzed as described in the text. Electrophoresis was from left to right.
(_____) ^{3}H radio-labeled protein from free polysomes.
(- - -) ^{14}C radio-labeled protein from membrane bound polysomes.

protein composition of polysomes was also observed when the cultures were treated in vivo with 200 μg/ml of puromycin for 1 minute at 35°C.

In order to eliminate the possibility that the extra protein bands of the free and the bound polysomes are the result of contamination by some soluble protein, the following experiment was designed. A cell extract containing membrane bound polysomes was mixed with labeled soluble protein (7×10^6 cpm) and a cell extract containing free polysomes was mixed with labeled detergent solubilized protein (5×10^6 cpm). The polysomes of both mixtures were purified and contained 7×10^2 cpm and 3×10^2 cpm respectively. When the proteins of those polysomes were analyzed by gel electrophoresis, the same patterns were obtained as shown in Fig. 2 for bound and free polysomes and no distinct peaks of label were found.

Discussion

Evidence has been presented which shows two differences between the protein of free and bound polysomes. Brief treatment of the chick embryo cells with puromycin in vivo, under conditions where nascent peptide chains are released, does not affect the presence of this protein fraction in the polysomes. It is also unlikely that the polysomes are contaminated unspecifically by soluble or detergent solubilized protein. High salt concentrations were reported to reduce greatly such non-specific interactions (10). Confirming this, it has been shown here that only 0.01% of labeled protein remains associated with bound and free polysomes after two cycles of sucrose gradient centrifugation. In addition, this low amount of contaminating protein does not move as a distinct band in electrophoresis.

Preliminary evidence indicates that the extra protein fraction unique to the free polysomes (band 2) is associated with the large ribosomal subunit (11).

The significance of having two types of ribosomes present in a cell may be several fold. First, the additional protein on free ribosomes may preclude the interaction of these ribosomes with membranes. Second, different ribosomes may be restricted to translate only certain types of m-RNA (1, 12) and

thus restrict the synthesis of some proteins to membrane-bound ribosomes and others to free ribosomes. Third, a difference in ribosome structure could also be the molecular basis for a differential cytoplasmic control of protein synthesis.

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