EUKARYOTIC RIBOSOMAL PROTEINS. TWO-DIMENSIONAL ELECTROPHORETIC STUDIES

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1. Introduction

Although some of the ribosomal proteins are believed to be different from species to species, they are thought to be identical within any one species [1–6]. It has also been reported that ribosomes, even from the same tissue, are heterogeneous in their protein content and/or function [7–13]. As for the number of proteins in an eukaryotic ribosome, it has been estimated to be similar [14] or higher [15] than in prokaryotic ribosomes.

Evidence is reported below which indicates, as expected, that ribosomes from two species differ in some of their protein components. But unexpectedly, some differences are also found in two different organs in the same species. Furthermore eukaryotic ribosomal proteins are shown to be more numerous than prokaryotic ones.

2. Experimental

2.1. *Preparation of rabbit reticulocyte ribosomes*

The ribosomes obtained by centrifugation [16–18] are suspended in (one-tenth of initial volume) buffer A (0.01 M tris-acetate, pH 7.2; 5 × 10^{-3} M Mg acetate; 0.3 M KCl). The ribosomes (20 to 25 mg of RNA estimated by the ratio of absorbance at 2600 Å and 2800 Å) are layered on a 1 × 20 cm column of DEAE cellulose (Whatman DE 11) regenerated by 0.2 M NaOH and 1 M HCl, and equilibrated by buffer A. The column is thoroughly washed free of hemoglobin and other proteins until the absorbance is back to the initial value.

The ribosomes are eluted by buffer B (0.01 M tris-acetate pH 7.2; 5 × 10^{-3} M Mg acetate; 0.55 M KCl), and collected by centrifugation at 150,000 *g*, 90 min [19–21].

2.2. *Preparation of ribosomes from rabbit liver and rat liver*

50 g of liver are homogeneized in 100 ml of buffer A [22]. Another 100 ml of buffer A is added and membranes are separated by centrifugation at 17,000 *g*, 10 min. The supernatant is centrifuged again (30,000 *g*, 60 min) to separate glycogen and membrane debris. Ribosomes are obtained by centrifugation of the supernatant at 150,000 *g*, 90 min. They are suspended in buffer A (one-tenth of initial volume). Ribosomal aggregates are eliminated by centrifugation 30,000 *g*, 60 min. As described above, the supernatant is layered on a DEAE cellulose column which is then thoroughly washed free of ferritin and other proteins with buffer A; the ribosomes are eluted with buffer B and collected by centrifugation. The use of sodium deoxycholate, which damages ribosomes [23] is avoided.

However, another method using sodium deoxycholate and described elsewhere [29] has been used to see whether the number of ribosomal proteins is identical.

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2.3. *Preparation of ribosomal proteins*

The proteins are extracted by 2 M LiCl dialyzed against water and lyophilized [24, 25]. The DOC ribosome pellet is treated overnight at 0° with 6 M urea and 4 M LiCl; after centrifugation (27,000 *g*, 15 min) the supernatant is dialyzed overnight against a large volume of 0.01 M HCl, the centrifuged and the supernatant, containing the ribosomal protein, is finally lyophilized.

2.4. *Electrophoresis*

Electrophoresis and staining of 1 or 2 mg of proteins are carried out according to Kaltschmidt and Wittmann [27] except for the following temperature during the run: 4°; first dimension: acrylamide concentration 4% pH 8.6 (constant voltage 200 V, initial intensity 5 mA/tube, run time: 14 hr); second dimension: acrylamide concentration 9% pH 4.6 (constant voltage 90 V, initial intensity 100 mA/gel, run time 17 hr.

3. Results and discussion

3.1. *Number of proteins in an eukaryotic ribosome*

The number of spots on the three electrophoretic gels indicates that the number of proteins in eukaryotic ribosomes purified on DEAE cellulose is higher than that in prokaryotic ribosomes [27]. 75 spots can be counted for rabbit liver ribosomes (fig. 2). This number may vary a little depending on the origin of the ribosome (see below) and the method of preparation. The possibility of a spot being an extra-ribosomal artifact may not be entirely excluded. However, the possibility is not very likely from the following considerations:

(1) Ribosomes purified on DEAE cellulose already loose about 30% of their protein content compared with washed ribosomes [20].

(2) A spot may be the result of an aggregation of two proteins, or conversely be derived from another one. This possibility is not very likely: it was observed in one only case in *E. coli* ribosomal proteins [28].

(3) Ribosomal proteins from one tissue (liver) prepared by two different methods, despite some dif-

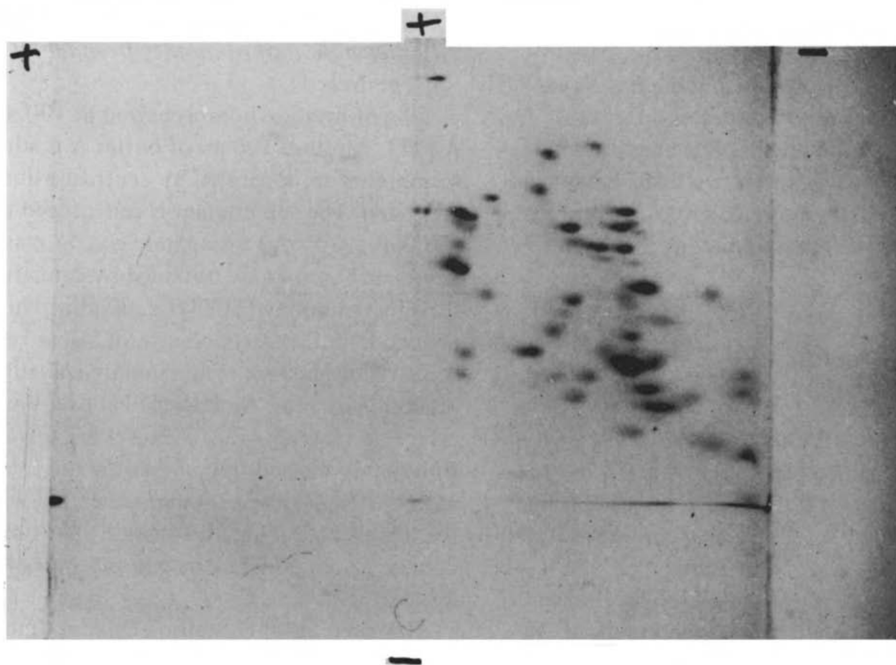

Fig. 1. Ribosomal proteins from rat liver: Two dimensional polyacrylamide gel electrophoresis according to the Kaltschmidt and Wittmann's methods.

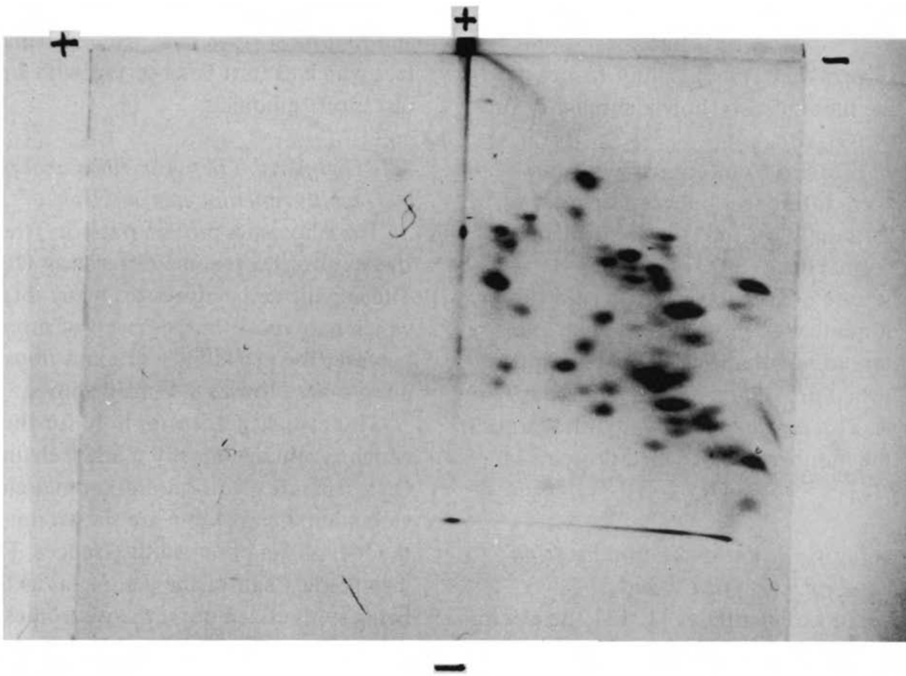

Fig. 2. Ribosomal proteins from rabbit liver.

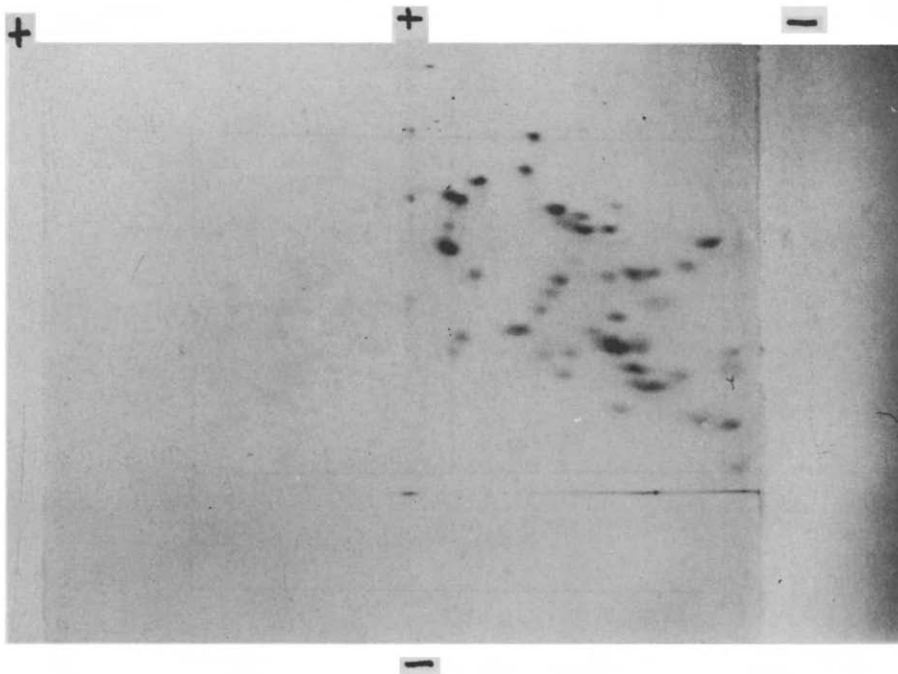

Fig. 3. Ribosomal proteins from rabbit reticulocytes.

ferences, both show a number of proteins higher than in *E. coli*.

(4) Ribosomal proteins from different tissues prepared by the same method also show a number of proteins higher than in *E. coli.*

(5) Ribosomal proteins from normal liver and hepatomas show no differences between themselves, although the hepatoma synthesizes many proteins different from normal liver [29].

So even in the case of artifacts which would slightly change the exact number of proteins in eukaryotic ribosomes, this would not change the conclusion that eukaryotic ribosomal proteins are more numerous than prokaryotic ones. This conclusion agrees with Warner's who estimated the number of ribosomal proteins in yeast to be 80 [15].

3.2. *Comparison between ribosomal proteins from rabbit liver and rat liver (figs. 1 and 2)*

As expected from earlier studies [1, 13] the electrophoretic patterns show some differences. The better resolution of the two-dimensional electrophoresis further shows that the general pattern is very similar and that most ribosomal proteins behave similarly, a fact which cannot be observed with one-dimensional disc electrophoresis.

3.3. *Comparison between ribosomal proteins from rabbit reticulocytes and liver*

The ribosomal protein patterns from these two tissues also shows some differences (figs. 2, 3 and 4). The hepatic cell synthesizes many different proteins which may result in the observed supplementary spots, however the probability of extra-ribosomal contamination is very low as discussed above.

This criticism does not hold for the reticulocytes which synthesize mostly α and β chain of hemoglobin. On a separate electrophoretic run α chain, β chain, globin and hemoglobin are shown not to migrate to the loci of the observed differences, They migrate into the left half of the gel. As far as the proteins being synthesized upon the ribosomes are concerned, their different lengths would make them invisible on the gel. The extra spots from reticulocytes ribosomes cannot therefore be considered as contaminants.

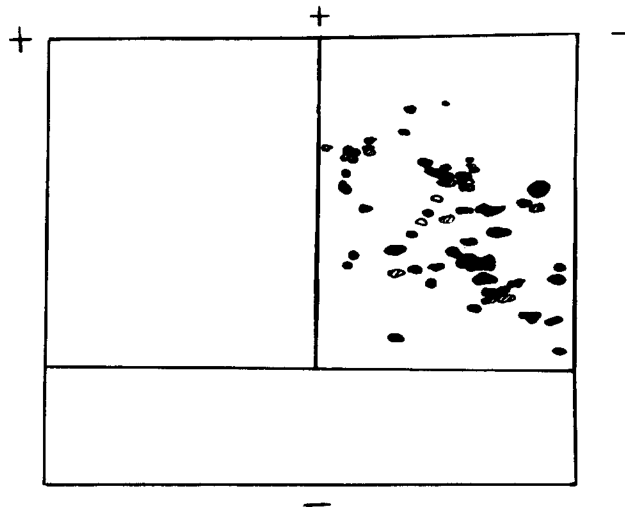

Fig. 4. Composite figure from figure 2 and 3, showing the observed differences. • Spots common to rabbit liver and reticulocytes. ◍ Spots found only in rabbit liver. ○ Spots found only in rabbit reticulocyte.

Di Girolamo and Cammarano [1] found no difference between rabbit reticulocyte and rabbit liver ribosomal proteins but they did show that the small subunit from these two tissues present different protein patterns. However they considered that the differences should be artifacts and may be due to some unknown enzymatic properties of the separated small subunits. Further investigation would probably show that the differences that we observed lie in the small subunit as found by Di Girolamo.

Acknowledgements

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