FREE AND MEMBRANE-BOUND RABBIT RETICULOCYTE RIBOSOMES. PROTEINS FROM THE LARGE AND THE SMALL SUBUNITS*

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1. Introduction

The presence of free and membrane-bound ribosomes seems to be a common feature of most mammalian cells. In reticulocytes these two types of ribosomes may be involved in the synthesis of globin [1–4]. The bound ribosomes in addition may be involved in the synthesis of non-globin proteins [5,6]. Recently it was shown that monosomes prepared from free and membrane-bound polysomes present some differences when their proteins were analysed by two-dimensional gel electrophoresis [7].

We have now determined that the two proteins, found in free polysomes only, are large subunit proteins and that out of the four proteins present in membrane-bound polysomes only, one is a large subunit protein, two are small subunit proteins and one is lost during dissociation of polysomes.

2. Materials and methods

2.1. *Preparation of subunits*

Free and membrane-bound polysomes were prepared as previously described [7]. Polysome pellets were resuspended in 8 ml of solution B (KCl 500 mM, $MgCl_2$ 2 mM, Tris 50 mM, pH 7.8) to a concentration of about 60 $O.D._{260\ nm}$ units per ml. Puromycin was then added to a final concentration of 0.1 μmol/$O.D._{260\ nm}$ unit (6 mM). The polysome suspension was then incubated 15 min at 0°C, and 15 min at 37°C. Then 1.5 ml of this solution was carefully layered on the top of a linear sucrose gradient 5–20% (w/v) in solution B. The gradient was centrifuged in a rotor SW 27 for 16 h at 16 000 rev/min. Fractions of 1 ml were collected and those corresponding to the subunits were pooled and dialysed overnight against distilled water. After lyophilisation the subunits were resuspended in 4 ml of water, dialysed again and lyophilised to remove completely traces of sucrose and KCl.

2.2. *Gel electrophoresis*

We have used the method originally described by Kaltschmidt and Wittmann [8] as modified by Howard and Traut [9] and Avital and Elson [10].

2.3. *Gel elution for molecular weight determination*

After electrophoresis gels were fixed and stained with Coomassie blue for only 30 min. After destaining spots were cut out and ground by passing through a syringe. Proteins were eluted from the ground gels in 1 or 2 ml of the SDS buffer used for disc electrophoresis [10]. After a 16 h dialysis against the same buffer diluted ten times the samples were lyophilised and resuspended in one tenth the initial volume before being analysed by SDS gel electrophoresis. Mol. wt determination was carried out as described by Weber and Osborn [11].

3. Results

Fig.1 shows the dissociation profile obtained with both free and membrane-bound ribosomes exposed

* Paper IV of the series. For paper II see [7].

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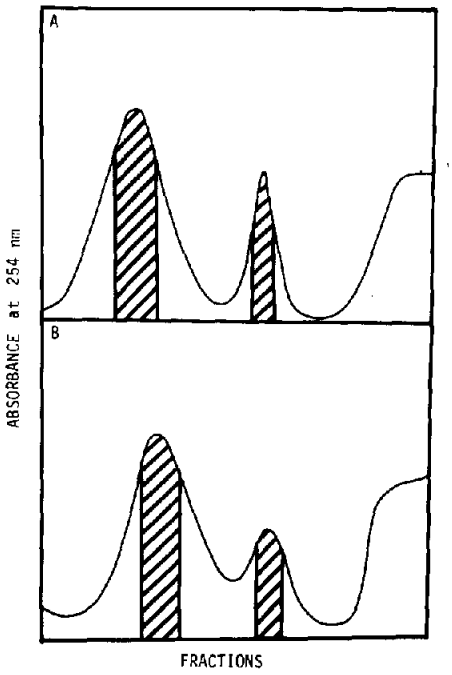

Fig.1. Separation of ribosomal subunits of rabbit reticulocytes. (A) Free ribosomes; (B) membrane-bound ribosomes. For experimental details see text.

to KCl-puromycin. To assure maximum purity only a few fractions were pooled under each peak. When the proteins of the two subunits from free ribosomes were analysed by gel electrophoresis, 42 proteins were found in the 60S subunit and 33 proteins in the 40S subunit. The two proteins, which were found previously in the free monosomes only, are proteins of the large subunit, L11 and L17 (fig.2 and fig.4). When membrane-bound subunits were analysed under the same conditions, it was found that among the proteins present in the membrane-bound monosomes only [7]: two belong to the 40S subunit, MS1 and MS2 (fig.3 and fig.4), one to the 60S subunit, ML1 (fig.2 and fig.4) and one is lost during dissociation, M4 (fig.4). The mol wt of five of these proteins were estimated by SDS gel electrophoresis and are given in table 1. The proteins were extracted from gels prepared from free and membrane-bound monosomes. Not enough of the ML1 protein was obtained to do an accurate molecular weight determination.

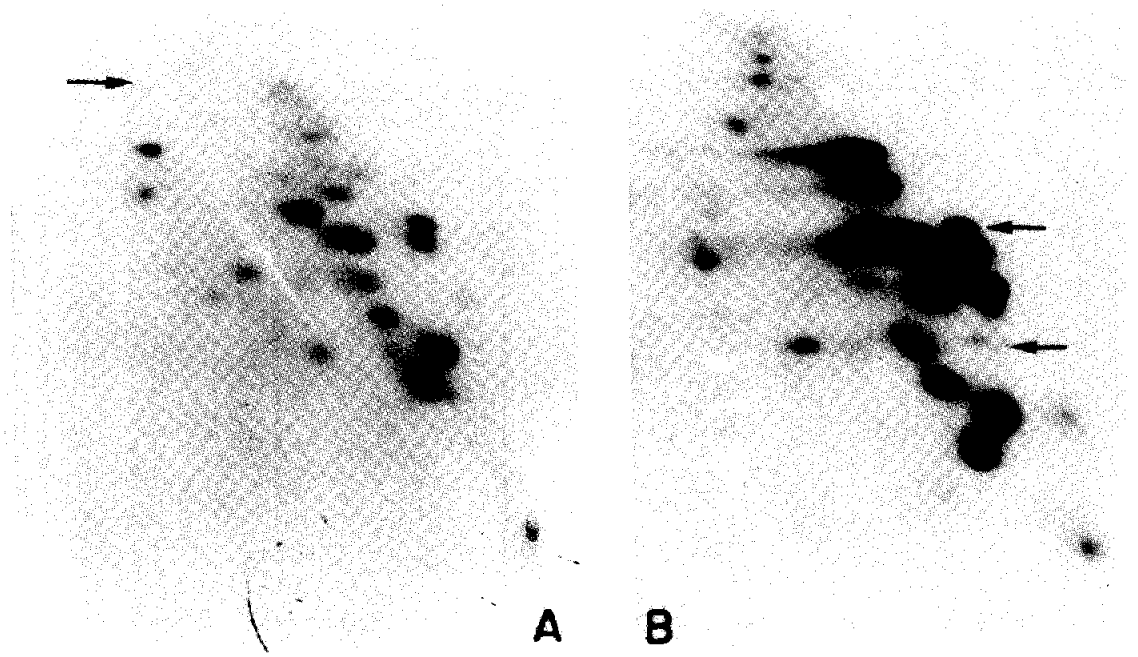

Fig.2. For caption to this figure, see p. 11.

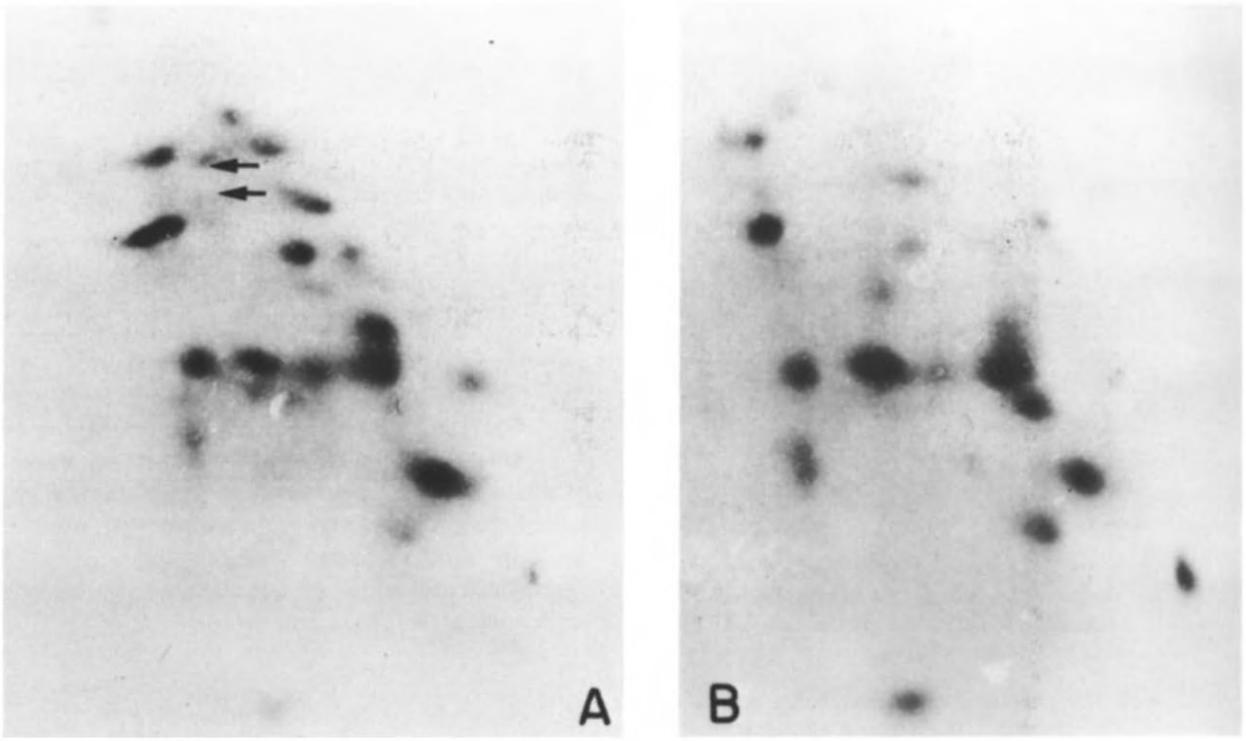

Fig.3.

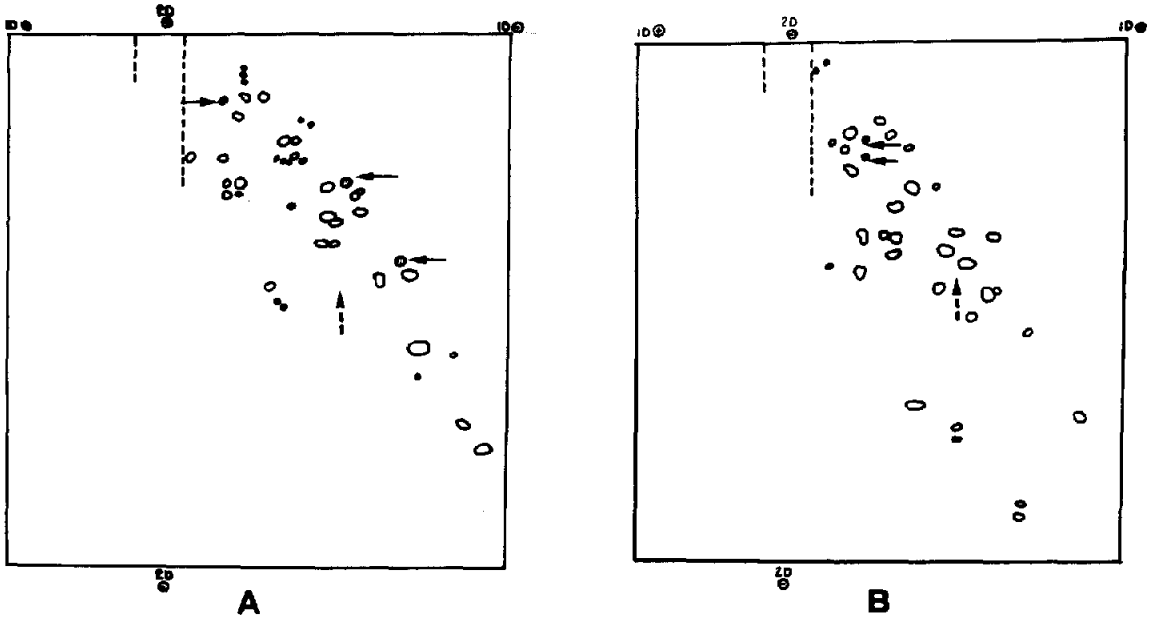

Fig.4.

Table 1
Molecular weight of some ribosomal proteins

Ribosomes		Mol. wt
Free	Membrane-bound	(in daltons)
L11		29 800
L17		20 000
	MS1	44 700
	MS2	46 800
	M4	19 500

The mol. wts were determined by the method of Weber and Osborn [10] using cytochrome *c*, myoglobin, pepsin, ovalbumin and serum albumin as standards. The maximum error was ± 700 daltons.

4. Discussion

The ribosomal protein patterns of the 40S and the 60S subunits obtained from free reticulocyte ribosomes are very similar to the patterns published by Chatterjee et al. [12]. We have found a total of 75 proteins in the two subunits, 42 in the large and 33 in the small subunit.

When we look at the differences already reported between free and bound monosomes [7] we see, as expected from the comparison made previously with Chatterjee's results, that the two proteins found in the free monosomes only are large subunit proteins L11 and L17. As far as the proteins found in bound monosomes only are concerned, M4, the one having the lowest mol. wt, is lost during dissociation, two are small subunit proteins and have high mol. wts. The fourth one, ML1, is a large subunit protein and from its position in the gel, one can deduce that it has also a high mol. wt. Recently Howard et al. [14] have determined the mol. wt of all the proteins of the subunits of free rabbit reticulocyte ribosomes. However, these authors have numbered their proteins along vertical lines instead of horizontal lines as done by Kaltschmidt and Wittmann [15], Sherton and Wool [16] and Chatterjee et al. [12]. Nevertheless it seems that our protein L11 (29 800) occupies the same position as proteins L30 (27 900) or L31 (28 800) of Howard et al. [14] and our protein L17 (20 000) the same position as their L33 (20 700).

It is improbable that the proteins found in the bound monosomes are degradation products of the proteins found only in the free monosomes. The higher mol. wts of the proteins MS1, MS2 and ML1 indicate that they cannot be derived from L11 and L18. These proteins can be considered as real ribosomal proteins, they are not lost during dissociation of the monosomes into subunits. The results were the same if membrane-bound monosomes were liberated from the membrane by puromycin treatment or by deoxycholate treatment.

These results in rabbit reticulocytes could support Tata's recent hypothesis [13] that topographic segregation of ribosomes involved in the synthesis of different proteins could be based on structural differences. On the other hand the small number of differ-

Fig.2. Two-dimensional gel electrophoretograms of ribosomal proteins from rabbit reticulocyte membrane-bound large subunit (A) and free large subunit (B). Some spots, clearly visible to the eye, are barely visible in the photograph. The arrows indicate protein differences between free and membrane-bound subunits. (A) ML1, found only in the membrane-bound large subunit. (B) Proteins found only in the free large subunit, starting from the top: 1st arrow, L 11; second arrow, L 17.

Fig.3. Two-dimensional gel electrophoretograms of ribosomal proteins from rabbit reticulocyte membrane-bound small subunit (A) and free small subunit (B). The proteins found only in the membrane-bound small subunit are indicated with arrows; first arrow from the top, MS1; second arrow MS2.

Fig.4. Schematic of the two-dimensional gel electrophoretograms of ribosomal proteins from rabbit reticulocyte ribosomes prepared with tracing paper placed over the partially dehydrated gel. The arrows indicate protein differences between free and membrane-bound ribosomes. (A) Large subunit proteins. Horizontal arrows starting from the top: (1) protein ML1 present only in membrane-bound large subunit; (2) protein L11 and (3) protein L17 present only in free large subunits. The vertical arrow indicates the position in membrane-bound monosome of proteins M4 lost during dissociation. (B) Small subunit proteins. Horizontal arrows starting from the top: (1) protein MS1 and (2) protein MS2 present only in membrane-bound small subunits. Vertical arrow: position of protein M4 in monosomes.

ences, a few out of the 75 ribosomal proteins, make it unlikely that the free and bound reticulocytes ribosomes come from two different ribosome pools. These differences are probably the result of transformation occurring at the time of binding of the ribosomes to the cell membrane or when the bound ribosomes are liberated from the membrane.

Acknowledgements

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