Short Communications

The Binding of Polyribosomes to Smooth and Rough Endoplasmic-Reticulum Membranes

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In vitro the binding of polyribosomes to smooth endoplasmic-reticulum membranes is more sensitive to ionic strength than is the binding to rough endoplasmic-reticulum membranes. Polyribosomes from the free and membrane-bound fractions bind with equal efficiency to endoplasmic-reticulum membranes.

There is strong evidence that proteins to be secreted from liver are made on polyribosomes attached to rough endoplasmic reticulum (see, e.g., Ganoza *et al.*, 1965; Takagi & Ogata, 1968; Ganoza & Williams, 1969; Redman, 1969; Hicks *et al.*, 1969; Takagi *et al.*, 1970). It is widely accepted that proteins to be retained within tissues, particularly the soluble tissue proteins, are made on free ribosomes (see the same references), although there are many reports that run counter to this generalization (see, e.g., Pitot & Jost, 1968; Ragnotti *et al.*, 1969; Takagi *et al.*, 1970; Puro & Richter, 1971). There is very little evidence on the site of synthesis of membrane proteins (Ragnotti *et al.*, 1969). However, the wide agreement that secreted proteins are made on membrane-bound ribosomes makes necessary a mechanism for segregating the appropriate mRNA molecules on to the correct sites for translation.

Reports that polyribosomes isolated from rough endoplasmic-reticulum membranes reattach more efficiently than do free polyribosomes both to smooth endoplasmic-reticulum membranes (Shires *et al.*, 1971) and to rough membranes stripped of endogenous ribosomes with 2M-LiCl (Scott-Burden & Hawtrey, 1971) appeared to provide such a mechanism. However, our own observations and those made by Nolan & Munro (1972) showed no differences between the two classes of polyribosomes. Also, the report that polyribosomes bind at all to smooth endoplasmic-reticulum membranes (Shires *et al.*, 1971) was contrary to results obtained in previous studies in which binding was assayed by similar direct methods (Suss *et al.*, 1966; Burka & Schickling, 1970; Ragland *et al.*, 1971).

We have therefore re-examined the binding of polyribosomes from the free and the membrane-bound fractions to smooth and rough endoplasmic-reticulum membranes. We report that binding of polyribosomes to smooth endoplasmic-reticulum membranes is very much more sensitive than is binding to rough membranes to changes in the ionic

strength of the incubation buffer. We also show that we cannot confirm in any of the experimental conditions tested the reports that polyribosomes from rough endoplasmic reticulum bind to membranes more efficiently than do free polyribosomes.

Materials and methods

Animals. Male Wistar rats, of weight 120-150g, were obtained from High Oak Ranch, Goodwood, Ont., Canada. They were maintained on standard laboratory diet and starved overnight with full access to water before use. Liver preparations used in the experiment indicated in Table 1 were essentially as described for preparations from mouse liver (Rolleston, 1972). Total liver polyribosomes were prepared as described for total liver ribosomes (Rolleston, 1972) with the exception that centrifugation of the deoxycholate-treated postmitochondrial supernatant was at 165000g (r_{av} , 5.9cm) for 30 min. Preparations used for the experiment indicated in Table 2 were as described by Scott-Burden & Hawtrey (1971) and Shires *et al.* (1971) with the modifications noted below. For preparation of labelled ribosomes, rats were injected with [3 H]orotic acid (500 μ Ci; specific radioactivity >15 Ci/mmol) and starved overnight.

Materials. Deoxycholic acid was purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A., and was recrystallized from ethanol before use. It was dispensed from a 10% stock solution of the sodium salt at pH 8.0. The sources of all other materials used have already been described (Rolleston, 1972).

Assays for binding. Apart from the different sources of the chemicals and animals used, experiments to repeat work described in previous reports (Scott-Burden & Hawtrey, 1971; Shires *et al.*, 1971) were as described in their experimental sections with the following exceptions. The methods used by Shires *et al.* (1971) were altered as follows: polyribosomes were labelled with 3 H rather than with 32 P; the

Beckman SW50.1 rotor was used at 189000g (r_{av} , 8.35cm) for 15h to separate membrane-bound from free ribosomes in the assay of binding; the membrane-containing layer was then collected by aspiration, diluted at least threefold with the same buffer as used in the assay and centrifuged at 114000g (r_{av} , 6.4cm) for 20min in the Beckman 40.3 rotor. The sedimented membranes were dissolved in 0.2% sodium dodecyl sulphate and their radioactivities counted as described previously (Rolleston, 1972). Results of the binding assay are expressed as percentages of the label in ribosomes added to the incubation mixture bound to membranes; this differs from the method of Shires *et al.* (1971), in which binding was expressed as the percentage of total radioactivity recovered in the membrane layer plus the sedimented free ribosomes. In our hands the use of the latter method of calculating binding of polyribosomes gave values for percentage binding up to twice those of the former. The methods used by Scott-Burden & Hawtrey (1971) were altered only in that centrifugation of the deoxycholate-treated rough endoplasmic-reticulum fraction to collect ribosomes was at 165000g (r_{av} , 5.9cm) for 2h. All other methods used have already been described (Rolleston, 1972).

Results and discussion

Table 1 presents a comparison of the binding of polyribosomes and 60S ribosomal subunits obtained by deoxycholate treatment of a postmitochondrial supernatant (i.e. containing free and bound poly-

ribosomes) to smooth and rough endoplasmic-reticulum membranes at pH 7.1 and 7.8 and in the presence of 25 mM- and 100 mM-KCl. Mouse preparations were used. Binding of polyribosomes and 60S ribosomal subunits to rough endoplasmic-reticulum membranes was generally greater than to smooth ($P < 0.01$). At both pH values binding of polyribosomes and 60S subunits to smooth endoplasmic reticulum was decreased to very low values ($P < 0.01$) by increasing the KCl concentration from 25 to 100 mM. The same change in KCl concentration decreased the binding of polyribosomes to rough endoplasmic-reticulum membranes by 50% at pH 7.8, but had no significant effect either on polyribosomes at pH 7.1 or on 60S subunits at either pH value. These results lead to the conclusion that binding of ribosomes to smooth membranes is by a mechanism different from that of the binding to membranes from rough endoplasmic reticulum, and raised the possibility that the former is largely an artifact of low ionic strength.

It has already been reported that 60S subunits from membrane-bound and from free ribosomes bind with high and equal efficiency to rough endoplasmic-reticulum membranes (Rolleston, 1972). In part this supports the observation by Baglioni *et al.* (1971) that, in intact cells, newly made 60S subunits bind to rough endoplasmic-reticulum membranes independently of the other components of polyribosomes, and suggests that *in vitro* binding of polyribosomes to membranes occurs merely because the binding site on the 60S subunit is not masked in polyribosomes.

Table 1. *Effect of pH and KCl concentration on the binding of mouse polyribosomes and 60S subunits to smooth and rough endoplasmic-reticulum membranes*

Rough endoplasmic reticulum was prepared from mouse liver and stripped of its endogenous ribosomes with KCl and puromycin, and smooth endoplasmic reticulum, cell sap and ^3H -labelled polyribosomes and 60S subunits were prepared as described by Rolleston (1972), with the only exception that membranes and cell sap were prepared throughout in medium at the same pH as used in the final assay. Subunits and polyribosomes were from the total liver fraction. Binding of polyribosomes was assayed as described by Ragland *et al.* (1971) with the modifications noted in the text under 'Materials and methods'. Binding of 60S subunits was as described by Rolleston (1972). The medium used in the binding assay was 50 mM-Tris-HCl buffer, at the pH value indicated, containing 25 mM- or 100 mM-KCl as indicated, 5 mM-MgCl₂ and 0.25 M-sucrose. Incubations were carried out at 37°C. Results are expressed as the percentages of added label bound to the endoplasmic-reticulum membranes, and are means $\pm$ S.D. for five independent experiments.

Incubation conditions		Ribosomes ... Endoplasmic-reticulum preparation ...	Binding (%)				
			Polyribosomes		60S subunits		
			Rough	Smooth	Rough	Smooth	
pH	Concn. of KCl (mM)						
7.1	25		64 ± 10	38 ± 12	56 ± 6	49 ± 6	
7.1	100		51 ± 9	10 ± 6	62 ± 7	23 ± 5	
7.8	25		60 ± 5	27 ± 14	61 ± 10	23 ± 1	
7.8	100		32 ± 4	3 ± 1	59 ± 3	14 ± 8	

Table 2. *Binding of polyribosomes from free and membrane-bound ribosomal fractions to smooth endoplasmic-reticulum membranes and to rough endoplasmic-reticulum membranes stripped of their endogenous ribosomes with 2M-LiCl*

Expt. A: assays were performed as described by Shires *et al.* (1971) with the modifications noted in the text under 'Materials and methods'. Expt. B: assays were performed as described by Scott-Burden & Hawtrey (1971) with the modification noted in the text under 'Materials and methods'. Results are expressed as the percentages of added label bound to endoplasmic-reticulum membranes, and are means $\pm$ S.D. for the numbers of independent experiments indicated.

Expt.	No. of observations	Source of polyribosomes ...	Binding (%)	
			Free	Bound
A	6		36 $\pm$ 2	40 $\pm$ 4
B	3		65 $\pm$ 2	63 $\pm$ 3

However, the observation that attachment of polyribosomes to rough endoplasmic-reticulum membranes is decreased by 50% when the KCl concentration is increased from 25 to 100mM at pH7.8, whereas the binding of 60S subunits is not affected (Table 1), suggests that the other components of polyribosomes may partially mask the site on 60S subunits that attaches to the membranes.

It has been reported that, in experiments with rats, polyribosomes from rough endoplasmic reticulum bind more efficiently than do free polyribosomes both to smooth endoplasmic-reticulum membranes (Shires *et al.*, 1971) and to rough endoplasmic-reticulum membranes (Scott-Burden & Hawtrey, 1971). We have repeated both experiments, using rats, under conditions very close to those reported. In both cases we have observed no difference between the two groups of polyribosomes (Table 2). Similarly, no differences were observed between free and membrane-bound polyribosomes in attachment to rough or smooth endoplasmic-reticulum membranes in the presence of 100mM-KCl at pH7.1 and 7.8 in either mouse or rat preparations (results not shown). We have no explanation for the differences between our observations and those made by the previous workers (Scott-Burden & Hawtrey, 1971; Shires *et al.*, 1971). Our observation that there is no difference between the two groups of ribosomes supports that made by Nolan & Munro (1972).

The observation that polyribosomes isolated from the free and membrane-bound fractions bind with equal efficiency to rough endoplasmic membranes indicates that ribosomes are not directed to membranes by components present in the polyribosomes as isolated. But it does not rule out the possibility, suggested by Milstein *et al.* (1972), that an *N*-terminal sequence, which is cleaved from the nascent peptide by a membrane-bound enzyme, directs the ribosomes to the membrane. However, such a mechanism for specific direction of certain ribosomes to the membranes would imply a very strong interaction between the *N*-terminal sequence involved and the membranes.

This strong interaction would be required to influence significantly the already very efficient binding of 60S ribosomal subunits to membranes in the absence of mRNA, 40S subunits and nascent peptides (Baglioni *et al.*, 1971; Rolleston, 1972).

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