A Comparative Study of Ribonuclease Hydrolysis of Rat Brain-Cortex and Liver Membrane-Bound Ribosomes

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Because it has been proposed that the ribosome-membrane interaction is different in endoplasmic reticulum derived from a non-secretory and secretory cell we undertook a study to determine whether attachment of the ribosome to the membrane involved ribosomal RNA and if the rRNA in ribosomes derived from the two classes of cell possessed an altered susceptibility to RNAase (ribonuclease) hydrolysis. We found that brain ribosomes appeared to possess more regions accessible to nuclease attack, independent of whether a sequence-dependent RNAase (T_1) or a sterically hindered RNAase bound to Enzite polymer was employed. These results were independent of whether the ribosomes were membrane-bound or detached from the endoplasmic reticulum membranes, but at high RNAase concentration these differences became negligible. No conclusions, however, could be drawn as to whether ribosomal RNA is involved in the attachment of the ribosome to the endoplasmic reticulum membrane, because of the presence of endogenous membrane-associated RNAases. Analysis of the rRNA fragments by polyacrylamide-gel electrophoresis suggests that the sites available for attack by low concentrations of nuclease in bound-ribosomes derived from brain cortex are different from those of liver.

It appears that nascent protein chains synthesized by the membrane-bound ribosomes in a secretory tissue (liver) traverse the endoplasmic reticulum membrane. This, however, does not hold true for proteins synthesized by non-secretory tissues (brain cortex, muscle) (Andrews & Tata, 1968, 1971) and differences in the ribosome-membrane interaction were proposed to explain the observed properties of the two classes of tissue.

Early experiments (Dingman & Sporn, 1962) showed that the ribosomal RNA extracted from brain and liver microsomes (microsomal material) possess similar properties with respect to u.v. spectra, thermal hypochromicity and kinetics of RNAase* hydrolysis, although small differences which may have been experimentally significant, were observed in the thermal 'melting' properties. Recently, McInnes (1972) showed differences in the composition of ribosomal proteins derived from brain and liver ribosomes.

It therefore appeared justifiable to investigate whether the rRNA in brain and liver ribosomes differed with respect to its susceptibility to a variety of RNAases. Such an approach might also reveal whether the RNA component of the ribosome is involved in the ribosome-membrane interaction: this might be shown by an altered accessibility of the rRNA to hydrolysis when the ribosomes are attached

to the endoplasmic reticulum membrane. A study was therefore commenced employing two nucleases (pancreatic RNAase A and RNAase T_1) with different sequence specificity to study the relative importance of RNA sequence, and an RNAase A bound to cellulose (Enzite) to determine how the accessibility of the rRNA was governed by the conformation of the ribosome.

Materials and Methods

The methods used to prepare 'heavy' endoplasmic reticulum fractions of brain and liver were those described by Andrews & Tata (1968) except for the following slight modifications. Four male Sprague-Dawley rats (300–400g) were used for each liver preparation and twelve to fifteen animals for each brain preparation. The homogenate medium for the liver was similar to that described by Andrews & Tata (1968) except that the buffer for homogenization was 0.25M-sucrose – 25mM-KCl – 10mM-MgCl₂ – 50mM-Tris-HCl, pH 7.5 (0.25M-sucrose – TKM buffer), and a 1.3M-sucrose barrier (as described by Tata & Williams-Ashman, 1967) was used in the first stage of fractionation of the microsomal pellet. The brain homogenate was also prepared by blending the brain-cortex tissue in 0.25M-sucrose – 100mM-KCl – 10mM-MgCl₂ – 50mM-Tris-HCl, pH 7.4, in a Waring Blender for 3 min.

* Abbreviation: RNAase, ribonuclease.

Free polyribosomes from liver were prepared from the 2.0M-sucrose layer of the last barrier-centrifugation. They were dialysed against the 0.25M-sucrose-TKM buffer described above for homogenization (the suspensions had the following absorbance ratios: $E_{260}/E_{240} \sim 1.2-1.3$ and $E_{260}/E_{280} \sim 1.7-1.8$).

Detached polyribosomes from both tissues were prepared by deoxycholate treatment [1% (w/v) for liver and 2% (w/v) for brain] of the heavy endoplasmic reticulum fraction in the presence of post-microsomal cell sap (an RNAase inhibitor) followed by centrifugation over a two-step barrier of 1.5M-sucrose and 2.0M-sucrose at 100000g for 14h in an SW40 rotor. The two-step barrier procedure resulted in the deoxycholate layer forming between the 1.5M- and 2.0M-sucrose layers with a polyribosome fraction possessing good spectral characteristics ($E_{260}/E_{240} 1.3-1.4$, $E_{260}/E_{280} 1.8-1.9$) being found in the 1.5M-sucrose layer. This method was especially useful when preparing ribosomes from the brain, which gave very low yields because: (a) the amount of RNA initially present attached to the brain endoplasmic reticulum was low (RNA content $\sim 120\mu\text{g}$ of RNA/mg of protein, cf. liver 300–400 μg of RNA/mg of total protein) and (b) deoxycholate treatment solubilized only part of the brain endoplasmic reticulum membrane. Ribosomes were also prepared from brain by treatment with 1% (w/v) deoxycholate and gave similar results to those prepared with 2% (w/v) detergent; the latter technique, however, resulted in a greater yield and was therefore used. These polyribosomes had RNA/protein ratios of 0.6 ± 0.1 and 0.7 ± 0.1 for the brain and liver respectively; these values are similar to those reported by Andrews & Tata (1971).

Labelled polyribosomes were prepared as above but with liver tissue derived from a rat given 200 μCi of [^3H]orotic acid (23 Ci/mmol, Amersham/Searle, Des Plaines, Ill., U.S.A.) per 100g body weight intraperitoneally 14h before death. The rat was fed before being killed to avoid decreased RNA synthesis (Blobel & Potter, 1967).

Chemical determinations

Protein was determined as described by Lowry *et al.* (1951) with crystalline bovine serum albumin as the standard. Membrane associated RNA was determined as described by Schneider (1957) and ribosomal RNA from the E_{260} (Munro & Fleck, 1966). Membrane RNA was determined by the method of Seifert & Remmer (1971).

Radioactive ribosomal RNA was hydrolysed with 0.3M-KOH for 18h at 37°C and then neutralized with perchloric acid. A synthetic equimolar mixture of AMP, UMP, GMP and CMP was added and the bases were separated by paper electrophoresis as described by Markham & Smith (1952). The spots were

cut out, the nucleotides eluted with 0.1M-HCl and counted for radioactivity with Bray's (1960) solution.

RNAase treatment

RNAase A and T_1 were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.) and RNAase A coupled to cellulose (Enzite) was from Miles (Kankakee, Ill., U.S.A.). RNAase A, T_1 and RNAase-Enzite were assayed by determining the number of E_{260} units released from a solution of yeast RNA (1mg/ml, Sigma Cat. No. 6750) at 37°C for 20min. The specific activities of the RNAase A and T_1 were 8.7×10^4 and 2.2×10^4 units/mg of RNAase, respectively. Two preparations of Enzite-RNAase were employed, the first possessing a specific activity of 9.1 and the second, 15.3 units/mg of RNAase.

RNAase treatment of the endoplasmic reticulum membrane-ribosome complex was performed at 37°C in 0.25M-sucrose–100mM-KCl–10mM-MgCl₂–50mM-Tris–HCl, pH 7.5 buffer, with a variable RNA/RNAase ratio. Each membrane-bound ribosome preparation was incubated alone because of the presence of variable amounts of endogenous nuclease and the amount of acid-soluble material thus liberated was subtracted from that liberated in the presence of added RNAase. The amount released with the membranes varied and was normally 20–40% of the amount liberated by the exogenous RNAase; detached polyribosomes possessed an endogenous RNAase that liberated 1–10% of that liberated by the added nuclease. The reaction was stopped at various times by adding trichloroacetic acid–uranyl acetate to final concentrations of 5% and 0.01% (w/v) respectively. The tubes were left overnight at 4°C, centrifuged at 1000g for 15min and the E_{260} of the supernatants was read in a Gilford 2400 spectrophotometer. If the acid-soluble material was labelled, 1ml was added to 10ml of Bray's (1960) solution and counted for radioactivity. The specific radioactivities of the ribosomal RNA were determined after digestion with NCS solubilizer (Nuclear Chicago, Ill., U.S.A.) or extraction by trichloroacetic acid at 90°C.

RNAase hydrolysis of the polyribosome samples was performed as for the membrane fractions except that, to aid precipitation of the polynucleotides and polypeptides, each portion (containing 50 or 100 μg of RNA) of the reaction mixture had 50 μg of bovine serum albumin added before precipitation with the unlabelled trichloroacetic acid–uranyl acetate solution.

Polyacrylamide-gel electrophoresis

Gels (5%, w/v) were prepared by the method of Peacock & Dingman (1967). Electrophoresis was performed in 10cm $\times$ 0.7cm tubes with gel lengths of approximately 7cm. A current of 2–3mA was em-

ployed per gel and the run was continued until the Bromophenol Blue marker migrated 3.5–4.0 cm. A (yeast) tRNA marker was always run in parallel and was found to migrate further than the tracking dye. Gels were stained with Methylene Blue as described by Peacock & Dingman (1967) and scanned by using a Joyce-Loebl densitometer IIIC with a red filter (5-042).

RNA samples were prepared from the bound ribosomes by phenol extraction employing sodium dodecyl sulphate to solubilize the membrane and ribosomal proteins (Peacock & Dingman, 1967). Before precipitation with ethanol the RNA solutions were dialysed under pressure with a Diaflo Apparatus (Amicon Co., Lexington, Mass., U.S.A.) with a PM 10 filter. The precipitated RNA was washed with diethyl ether, dried and then dissolved in 20% sucrose–0.004% Bromophenol Blue solution before application to the gel.

Sucrose gradients

The sample (2 ml) was layered on a 15–30% (w/v) sucrose gradient in TKM buffer. Centrifugation was at 88000g in a SW40 rotor for 14 h at 4°C; fractions of volume 0.5 ml were collected and their E_{260} was determined. The polyribosomal pellet was resuspended in 0.5 ml of buffer and its absorbance determined.

Results

We determined the amount of oligonucleotide material liberated after various times of incubation of the endoplasmic reticulum membrane-ribosome complex with low RNAase concentrations. Before using such a technique with the ribosome-membrane complex, it was necessary to establish that: (a) the detached polyribosomes were polyribosomes and did not contain degraded material or subunits and (b) that the E_{260} was an adequate measurement of the amount of oligonucleotide material liberated by RNAase treatment of the endoplasmic reticulum membrane. The sucrose gradient profiles of the control polyribosomes (Fig. 1) show that there is very little non-ribosomal material present in either sample and that virtually all the detached ribosomal material is in the form of polyribosomes. A more sophisticated technique than measurement of the E_{260} is to label the RNA phosphate groups with ^{32}P and then determine the amount of acid-soluble radioactivity released after enzymic hydrolysis. Unfortunately this is not possible with the endoplasmic reticulum, because the membrane phospholipids are labelled in addition to the ribosomes and thus any radioactivity liberated may be due to phospholipid as well as oligonucleotide. Orotic acid, a pyrimidine precursor that labels only RNA, will preferentially label ribosomal RNA if a long enough time elapses after injection. The heavy endoplasmic reticulum fraction thus

labelled was treated with low concentrations of pancreatic A, Enzite-RNAase and T_1 RNAase. The amount of RNA released, expressed in terms of either radioactivity or absorbance, results in hydrolysis curves of a similar shape (Fig. 2), an effect especially noticeable with pancreatic RNAase and Enzite-RNAase. Separation of the bases of the labelled RNA showed that the amount of radioactivity that migrated with the added UMP was about 20% more than that found with CMP; however rRNA contains twice as much CMP as UMP (Spirin & Gavrilo, 1969), and thus the specific radioactivity of the UMP is twice to thrice that of CMP. The amount of radioactivity will therefore be greater than the E_{260} measurements indicate if the released oligonucleotides contain more uracil residues than the untreated ribosomes. This is expected with pancreatic A and T_1 RNAase treatment, as hydrolysis of either the 18S or 28S rat liver RNAase T_1 and A results in the preferential loss of the adenine and uracil bases (Delihias, 1967). We therefore concluded that monitoring the E_{260} was an adequate method to determine the degree of RNAase hydrolysis of membrane-bound and detached ribosomes.

Fig. 1. Sedimentation profiles of the detached-bound polyribosomes from brain cortex (●) and liver (○) in a 15–30% (w/v) sucrose gradient

Both samples contain some 80S monomers (the one from liver more than the one from brain cortex) but few subunits or degraded material. Some or all of the absorbance of the material at the top of the gradient is due to the deoxycholate-solubilized membranes. --- indicates the absorbance profile of heavy polyribosomal aggregates resuspended in 0.5 ml of TKM buffer.

Fig. 2. Radioactivity and E_{260} released from polyribosomes by incubation with RNAase

(a) Shows the % of the total radioactivity released from membrane-bound polyribosomes (Δ) and from free liver polyribosomes ($\bullet$), and the % of total E_{260} units released from membrane-bound (Δ) and free liver polyribosomes ($\circ$) after incubation at 37°C with a pancreatic RNAase/RNA ratio of 1:70 (w/w). (b) Shows the % of the total radioactivity (Δ , $\bullet$) and the % of total E_{260} units (Δ , $\circ$) released from membrane-bound polyribosomes after incubation at 37°C with Enzite-RNAase (specific activity 15.3 units/mg) (Δ , Δ) and T_1 RNAase ($\bullet$, $\circ$). The RNAase/RNA ratios were 66/1 and 1/70 (w/w) respectively.

Heavy endoplasmic reticulum membrane fractions from brain cortex and liver were incubated with various RNAases in the sucrose-TKM buffer from brain cortex, which contains a high concentration of KCl (100mM). Brain polyribosomes are more susceptible to dissociation than those from liver (Zomzely *et al.*, 1966) and, as the ribosomal subunit appears more susceptible to nuclease attack than the

complete unit (Cox, 1969; Pinder & Gratzer, 1972), all the data were obtained with the higher-ionic-strength buffer. However, use of the more commonly used low K^+ concentration buffer did not influence the amount of oligonucleotide material released from brain ribosomes.

The results of RNAase hydrolysis of the endoplasmic reticulum ribosome-membrane complex and the bound ribosomes removed from the membranes by deoxycholate treatment from both brain cortex and liver are presented in Tables 1 and 2. Hydrolysis of the brain ribosomes releases more oligonucleotide material than similar preparations from rat liver (Table 1). This result is independent of (a) whether or not the endogenous RNAase blank is subtracted and (b) the sequence or structural specificity of the RNAase employed. Detached ribosomes derived from brain cortex are also more susceptible to hydrolysis than those derived from the liver (Table 2), the difference being of the order of 100% (as observed with the membrane-bound ribosomes) except in the case of RNAase A, where there was a far smaller difference between the two species of ribosome. Note that large differences were observed with the polymer-bound RNAase A. If it is assumed that the sequence specificity of this polymer-bound enzyme remains unchanged, then a structural difference in the two classes of ribosome may explain these results, there being regions of RNA sequence accessible in the liver ribosome to RNAase A but buried and inaccessible to the polymer-bound enzyme. A similar difference is observed with the T_1 RNAase, which has a very marked RNA sequence specificity.

The possibility of the endoplasmic reticulum membrane of the liver containing RNAase inhibitors can also be ruled out because (a) hydrolysis of a mixture of membrane-bound liver and brain-cortex ribosomes resulted in the amount of RNA liberated being equal to the sum of the two samples hydrolysed independently and (b) the brain-cortex ribosomes detached from the membrane also show an increased susceptibility to RNAase. The actual amounts of RNA liberated in the membrane-bound and detached polyribosomes cannot be compared because of the endogenous RNAase in the former.

The difference between the two species of membrane-bound ribosome may be due to more membrane RNA (Gardner & Hoagland, 1968) being associated with the brain endoplasmic reticulum which may be hydrolysed preferentially to the less accessible ribosomal RNA. This can be discounted because Shires *et al.* (1971) have shown that RNAase treatment (at far higher concentrations than employed here) of the liver endoplasmic reticulum membrane results in no decrease in the amount of membrane-associated RNA, only in a loss in the amount of rRNA. Secondly, the relative concentrations of membrane RNA and ribosomal RNA when determined for the two endo-

Table 1. *Amounts of acid-soluble material released by Enzite-RNAase, RNAase A and T₁ hydrolysis of membrane-bound ribosomes from the brain cortex and liver*

Results are % of total nucleotide material released into the supernatant by hydrolysis with Enzite-RNAase, pancreatic type A RNAase and T₁ RNAase with incubation periods of 120 min as measured by the E_{260} . The ratio of enzyme to ribosomal RNA was 50/1 (w/w) for Enzite-RNAase and 1/100 (w/w) for the A and T₁ RNAases. The total amount liberated refers to that resulting from exogenous plus endogenous RNAase and the difference is that liberated solely by exogenous RNAase. Errors are expressed as $\pm$ s.d. Values in parentheses refer to the number of determinations.

RNAase	Material released (%)			
	Membrane-bound ribosomes from brain cortex		Membrane-bound ribosomes from liver	
	Total	Difference	Total	Difference
Enzite-RNAase (450 units/mg of RNA)	17 $\pm$ 2 (3)	9 $\pm$ 1 (3)	7 $\pm$ 2 (3)	4 $\pm$ 1 (3)
Pancreatic RNAase (870 units/mg of RNA)	23 $\pm$ 2 (3)	13 $\pm$ 2 (3)	10 $\pm$ 1 (3)	7 $\pm$ 2 (3)
T ₁ RNAase (220 units/mg of RNA)	12 $\pm$ 2 (2)	6 $\pm$ 2 (2)	8 $\pm$ 1 (2)	4 $\pm$ 1 (2)

Table 2. *Amounts of acid-soluble material released by Enzite-RNAase, RNAase A and T₁ hydrolysis of detached membrane-bound ribosomes from the brain cortex and liver*

For details see Table 1.

RNAase	Material released (%)			
	Detached-ribosomes from brain-cortex endoplasmic reticulum		Detached-ribosomes from liver endoplasmic reticulum	
	Total	Difference	Total	Difference
Enzite-RNAase	13 $\pm$ 1 (2)	12 $\pm$ 1 (2)	7 $\pm$ 1 (2)	6 $\pm$ 1 (2)
Pancreatic RNAase A	15 $\pm$ 1 (4)	12 $\pm$ 1 (4)	12 $\pm$ 1 (4)	9 $\pm$ 1 (4)
T ₁ RNAase	10 $\pm$ 1 (2)	8 $\pm$ 1 (2)	5 $\pm$ 1 (2)	4 $\pm$ 1 (2)
			11 $\pm$ 1 (2)*	6 $\pm$ 1 (2)*

* These values refer to the amounts of acid-soluble material liberated from free polyribosomes from liver.

Table 3. *Effect of increasing concentrations of RNAase A on the amount of acid-soluble material liberated from the membrane-bound ribosomes*

The incubation medium is as described in the Materials and Methods section; the % of acid-soluble nucleotide liberated is calculated from the E_{260} of the acid-soluble material. The temperature was 37°C and the time of incubation 90 min. Errors are expressed as $\pm$ s.d. with the number of determinations given in parentheses.

RNAase A/ RNA ratio	Total nucleotide material released from membrane-bound brain-cortex ribosomes	Amount liberated with endogenous RNAase blank subtracted	Total nucleotide material released from membrane-bound liver ribosomes	Amount liberated with endogenous RNAase blank subtracted
700:1	13 $\pm$ 1 (4)	9 $\pm$ 1 (4)	6 $\pm$ 2 (4)	5 $\pm$ 2 (4)
100:1	22 $\pm$ 3 (4)	11 $\pm$ 1 (4)	10 $\pm$ 1 (4)	9 $\pm$ 1 (4)
10:1	22 $\pm$ 2 (2)	17 $\pm$ 2 (2)	23 $\pm$ 2 (2)	19 $\pm$ 2 (2)
10:1*	29 $\pm$ 4 (2)	24 $\pm$ 4 (2)	38 $\pm$ 4 (2)	32 $\pm$ 4 (2)

* Mg²⁺ concentration in incubation medium decreased from 10 to 5 mM.

Fig. 3. Photographs and densitometer traces of polyacrylamide gels

(a) Shows photographs of 5% (w/v) polyacrylamide gels of the non-treated membrane-bound RNA fractions from liver (i) and brain cortex (ii). Some 5S RNA is present in both fractions together with a little 4S RNA, the majority of the rRNA, however, does not enter the gel, showing lack of degradation. (b) Shows densitometer traces of the polyacrylamide gels of the ribosomal RNA fragments obtained after Enzite-RNAase digestion of membrane-bound polyribosomes from the liver (—) and brain cortex (---). The RNAase/RNA ratio was 1:1 (w/w) for 15 min at 37°C (RNAase specific activity 9.1 units/mg). Yeast tRNA and liver 5S RNA were used as molecular weight markers.

plasmic reticulum membrane preparations was similar; in fact the liver membranes appeared to contain slightly more RNA than the brain (Table 3). Thirdly, the gels (Fig. 3a) show no significant increase in 4S RNA material in RNA derived from the brain cortex endoplasmic reticulum and fourthly, detached

ribosomes show a difference similar to that of attached ribosomes.

The effect of increasing RNAase concentrations was determined with the membrane-bound ribosomes (Table 4). The differences observed at low RNAase/RNA ratios appeared to disappear at high RNAase concentrations and lowering the Mg^{2+} concentration resulted in the liver rRNA in the ribosome becoming more labile than the brain rRNA to RNAase A.

The membrane-bound ribosomes were treated with RNAase, extracted with phenol and then analysed by polyacrylamide-gel electrophoresis in 5% gels [which exclude both the 18 and 28S rRNA (Bishop *et al.*, 1967)]. Control (non-treated) samples contained a little 5S and 4S RNA (which enters the gel) but most of the RNA did not enter the gel, as expected for non-degraded rRNA (Fig. 3a).

Enzite-RNAase treatment of the membrane-bound ribosomes appeared to result in some detectable size differences in fragments from the two tissues. Treatment of liver ribosomes (Fig. 3b) produced a high (~500000)-molecular-weight band and four bands in the 100000 region, and similar treatment of brain ribosomes produced fragments of molecular weight in the region of 100000. These results suggest that the rRNA in brain ribosomes bound to the endoplasmic reticulum membrane possess sites susceptible to Enzite-RNAase attack that are different from those attached to liver ribosomes. Treatment of ribosomes with T_1 RNAase resulted in a similar series of fragments with both membrane preparations.

Conclusions

There are two points which can be made from this study. First, it is impossible to determine whether or not attachment of the ribosome to the membrane results in a significant amount of the rRNA becoming less accessible to attack by a sterically hindered nuclease, because of the high concentration of endogenous RNAase observed in both endoplasmic reticulum membrane preparations.

Secondly, all RNAase hydrolyses liberated more material from the detached-bound and bound ribosomes derived from the brain cortex than from the liver. This is not due to the presence of other membrane-associated RNAases and is only observable at low RNAase concentrations; at high RNAase/RNA ratios (1:10) these differences become negligible, probably because the hydrolysis makes hidden breaks accessible to nuclease attack (Cox, 1969).

Enzite-RNAase treatment of the bound ribosomes from the two tissues also produces a different pattern of fragments. T_1 RNAase treatment shows little difference between the two ribosome preparations, a result that confirms the observation of Gould *et al.* (1966) that an altered pattern of rRNA fragments

Table 4. *Relative amounts of membrane RNA and ribosomal RNA present in the endoplasmic reticulum membrane fraction derived from brain cortex and liver*

The determinations were performed as described in the Materials and Methods section. The errors are expressed as $\pm$ S.D.

Membrane sample	% of membrane RNA	% of ribosomal RNA
Endoplasmic reticulum from brain cortex	6 ± 4	94 ± 4
Endoplasmic reticulum from liver	18 ± 10	82 ± 10

was obtained with this nuclease only with ribosomal RNA derived from different species; also Higashi *et al.* (1972) found that this enzyme revealed little difference between rRNA from liver and kidney tissue, although it detected distinct differences between foetal and tumour tissue in comparison with adult tissue.

One important point is whether a ribosome attached to the membrane can be compared with a ribosome removed from the membrane by detergent treatment. We have attempted to answer this criticism by employing free, non-detergent-treated polyribosomes and have shown that a similar hydrolysis curve is produced (Fig. 2), as with the detergent-treated ribosome. In fact slightly more material is liberated with the free polyribosomes. This type of control is probably the best available at present, as any method used to remove ribosomes from the membrane such as citrate-phosphate buffers (Ragland *et al.*, 1971) will probably affect the ribosomal structure in an ill-defined manner. However, it must be remembered that minor differences in protein composition apparently exist between bound and free polyribosomes in chick embryo (Fridlender & Wettstein, 1970); also brain cortex ribosomal RNA species were methylated at different rates (Murthy, 1972), although no determinations were made to see if this was a large enough effect to alter the final base composition. Whether these minor compositional differences will result in a major structural difference in the ribosome appears somewhat unlikely.

We therefore conclude that the accessibility of the rRNA species of brain-cortex and liver ribosomes to low RNAase concentrations is different. These observed differences could be due to the different protein compositions of the ribosomes derived from the two tissues (McInnes, 1972) or an altered RNA-protein interaction caused by different rRNA conformations and/or sequence. The latter appears somewhat unlikely as no vast differences were seen in the kinetics of RNAase hydrolysis of RNA (Dingman & Sporn, 1962) and titration with a fluorescent dye, ethidium bromide; also, a comparison of the circular dichroism spectra of both classes of ribosomes showed no detectable differences (H. Simpkins, unpublished work).

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References

- Andrews, T. M. & Tata, J. R. (1968) *Biochem. Biophys. Res. Commun.* **32**, 1050-1056
- Andrews, T. M. & Tata, J. R. (1971) *Biochem. J.* **121**, 683-694
- Bishop, D. H. L., Claybrook, J. R. & Spiegelman, S. (1967) *J. Mol. Biol.* **26**, 373-387
- Blobel, G. & Potter, V. R. (1967) *J. Mol. Biol.* **26**, 279-292
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279-285
- Cox, R. A. (1969) *Biochem. J.* **114**, 753-767
- Delihias, N. (1967) *Biochemistry* **6**, 3356-3362
- Dingman, W. & Sporn, M. B. (1962) *Biochim. Biophys. Acta* **61**, 164-177
- Fridlender, B. R. & Wettstein, F. O. (1970) *Biochem. Biophys. Res. Commun.* **39**, 247-253
- Gardner, J. A. A. & Hoagland, M. B. (1968) *J. Biol. Chem.* **243**, 10-19
- Gould, H. S., Bonanou, S. & Kanagalingam, K. (1966) *J. Mol. Biol.* **22**, 397-399
- Higashi, K., Gotoh, S., Nishinaga, K. & Sakamoto, Y. (1972) *Biochim. Biophys. Acta* **262**, 320-327
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- Markham, R. & Smith, T. D. (1952) *Biochem. J.* **52**, 558-565
- McInnes, J. (1972) *J. Mol. Biol.* **65**, 157-161
- Munro, H. N. & Fleck, A. (1966) *Analyst (London)* **91**, 78-88
- Murthy, V. M. R. (1972) *J. Biol. Chem.* **247**, 1944-1950
- Peacock, A. C. & Dingman, C. W. (1967) *Biochemistry* **6**, 1818-1827
- Pinder, J. C. & Gratzner, W. B. (1972) *Eur. J. Biochem.* **26**, 73-80
- Ragland, W. L., Shires, T. K. & Pitot, H. C. (1971) *Biochem. J.* **121**, 271-278
- Schneider, W. C. (1957) *Methods Enzymol.* **3**, 680-684
- Seifert, J. & Remmer, H. (1971) *Biochem. Pharmacol.* **20**, 553-560
- Shires, T. K., Narurkar, L. & Pitot, H. C. (1971) *Biochem. J.* **125**, 67-79
- Spirin, A. S. & Gavrilova, L. P. (1969) *The Ribosome*, p. 42, Springer-Verlag, New York
- Tata, J. R. & Williams-Ashman, H. C. (1967) *Eur. J. Biochem.* **2**, 366-374
- Zomzely, C. F., Roberts, S., Brown, D. M. & Provost, C. (1966) *J. Mol. Biol.* **19**, 455-468