TWO CLASSES OF MEMBRANE-BOUND RIBOSOMES IN RAT LIVER CELLS AND THEIR ALBUMIN SYNTHESIZING ACTIVITY

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SUMMARY

Two classes of membrane-bound ribosomes were shown in rat liver cells. A small portion of them (loose ribosomes) was dissociated from the rough endoplasmic reticulum by RNase treatment, whereas the remainder still attached tightly to the membrane (tight ribosomes). Pulse-labeling experiment in vivo showed that they are both active in protein synthesis, but serum albumin is exclusively synthesized on tight ribosomes.

It is well known that liver cells contain two kinds of polysomes; free and membrane-bound polysomes. The latter was shown to synthesize secretory proteins, whereas the former to synthesize intracellular proteins.[1-4] Recently, however, a number of reports have provided evidence that bound polysomes synthesize some kinds of membrane proteins[5,6] and catalase, probably that in microbodies.[2,7] The variety of functions of bound polysomes suggested the diversity of their modes of interaction with the membrane. In fact, there have been some reports suggesting the presence of two types of bound ribosomes (loose and tight ribosomes) in animal cells.[8-10] It must be mentioned, however, that functional differences between these two types of ribosomes have not yet been clarified.

This communication describes the methods to fractionate these two kinds of bound ribosomes from rat liver and their

abilities to synthesize serum albumin, thus showing the functional differences of these two kinds of bound ribosomes.

METHODS

Rats of Wistar strain, weighing about 150 g and starved overnight, were injected with 50 μC of ^{3}H-leucine (150 C/M) or 5 μC of ^{14}C-leucine (331 C/M) through the portal vein.[2] The rats were decapitated after 1 min and the livers were excised within 30 sec. The postmitochondrial supernatant was prepared as described previously[4], using 0.25 M sucrose in TKM buffer (0.05 M Tris-HCl, pH 7.5, 0.025 M KCl and 5 mM $MgCl_2$) as homogenizing medium.

For the preparation of three types of liver ribosomes the modified method of Blobel and Potter[11] was employed: 6.5 ml of the postmitochondrial supernatant was layered over a discontinuous sucrose gradient with 2 M and 1.38 M sucrose in TKM buffer and centrifuged at 229,000 xg for 12 h. 2 M and 1.38 M sucrose solutions were prepared by mixing 2.3 M sucrose in TKM buffer with the freshly prepared postmicrosomal supernatant obtained by centrifugation of the postmitochondrial supernatant at 229,000 xg for 3 h. The 1.38 M sucrose layer containing rough endoplasmic reticulum was collected, diluted two-fold with TKM buffer and RNase was added to give a final concentration of 3 μg/ml. After standing at 0°C for 5 min, the membrane fraction was subjected to discontinuous gradient centrifugation as described above. Loose ribosomes released by RNase treatment were precipitated. The 1.38 M sucrose layer containing the tight ribosome-membrane complex was collected, diluted two-fold with TKM buffer and sodium deoxycholate was added to give a final concentration of 1 %. The mixture was then sub-

jected to the same discontinuous sucrose gradient centrifugation as above which precipitated tight ribosomes.

RESULTS AND DISCUSSION

Rosbash and Penman[5] suggested that loose ribosomes attached to the membrane via messenger RNA in HeLa cells, since they were dissociated by RNase treatment. In order to make search for such ribosomes in liver cells, effect of RNase on ribosome-membrane interaction was investigated. The distinct release of ribosomes from the membrane fraction was observed after RNase treatment, whereas an insignificant amount of them was obtained by centrifugation of the membrane fraction without RNase treatment (Table 1). The results strongly suggest the existence of ribosomes in liver cells with similar characteristics to loose ribosomes in HeLa cells, although their relative amounts to tight ribosomes are small in the case of liver cells.

Sucrose density gradient centrifugation revealed that loose and tight ribosomes prepared as described above contained only monomers and dimers owing to RNase treatment. It must be mentioned, however, that they had highly labeled nascent proteins and their specific radioactivity per absorption unit at 260 nm of ribosomes was comparable to that of free polysomes (Fig. 1). The results may indicate that most of these two types of bound ribosomes are present as polysomes in liver cells.

The pulse-labeled nascent proteins released from these three types of ribosomes were analyzed by SDS-acrylamide gel electrophoresis[12] to compare their size distributions (Fig. 2). They were all large enough to suggest synthesis on large polysomes. Fig. 2 also shows that the contamination of free

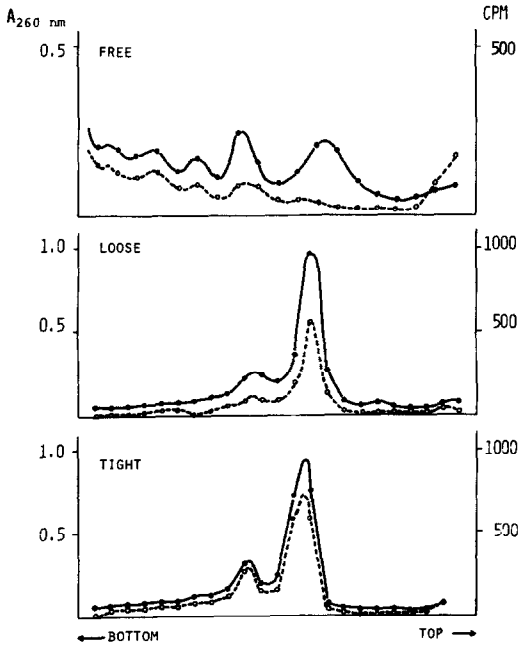

Fig. 1.

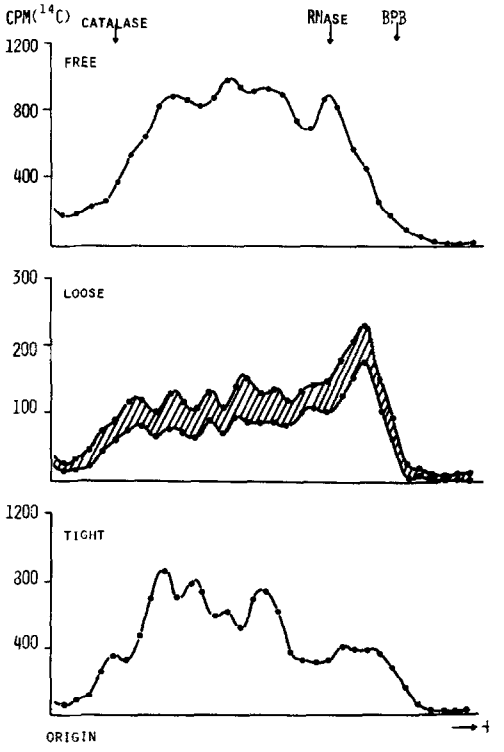

Fig. 2.

Fig. 1. Sedimentation profiles of three ribosomal preparations in sucrose gradient. 560 µg of free ribosomes, 280 µg of loose ribosomes and 250 µg of tight ribosomes suspended in 1 ml of 0.25 M sucrose in TKM were layered on 35 ml of 15-35 % (w/v) linear sucrose gradient in TKM and spun in a Spinco SW-27 rotor at 27,000 rpm for 4 h. The centrifugal tube was punctured at the bottom and the ultraviolet absorbance at 260 nm of the fractions of each 20 drops was measured. The 1 ml aliquot of each fraction was mixed with 8 ml of Triton scintillator (mixture of 3 volumes of Triton X-100 and 7 volumes of toluene containing 0.6 % 2,5-diphenyloxazole) and the radioactivity was measured in a Beckman LS-150 scintillation counter.

·——·——· Absorbance at 260 nm, o---o---o Radioactivity.

Fig. 2. SDS-acrylamide gel electrophoresis of nascent proteins. The mitochondrial supernatant obtained from a rat liver labeled with ^{14}C-leucine was mixed with free ribosomes from another rat liver labeled with ^{3}H-leucine. Free, loose and tight ribosomes were suspended in 0.1, 0.2 and 0.3 ml of 50 mM Tris pH 7.6, respectively. After the addition of EDTA (30 mM) and RNase (10 µg/ml), the suspension was incubated at 37°C for 15 min and 0°C overnight and centrifuged at 10,000 xg for 30 min. 0.1 ml of the supernatant was subjected to SDS-acrylamide gel electrophoresis as described by Weber and Osborn (12). The gels were stained and cut into 2 mm discs. The radioactivity of each disc was measured by the method of Basch (13) with a Beckman LS-150 scintillation counter.

Table 1. Effect of RNase on release of ribosomes from the membrane. Loose and tight ribosomes were prepared with or without RNase treatment of the membrane fraction. The amount of free ribosomes means their recoveries from the same preparations for loose and tight ribosomes. The amount of ribosomes was calculated from the absorbance at 260 nm and shown as mg recovery from g of liver.

membrane fraction treated		free	loose	tight
without RNase	0°C, 5 min	0.917	0.033	0.477
"	37°C, 5 min	0.898	0.049	0.468
with RNase (3 μg/ml)	0°C, 5 min	0.940	0.118	0.324
	37°C, 5 min	0.927	0.122	0.307

Table 2. Incorporation of ^{3}H-leucine into the serum albumin fraction of three classes of ribosomes. Three ribosomes obtained from 3 rats were suspended in 3 ml of 0.25 M sucrose in TKM. Nascent proteins were obtained by EDTA treatment followed by centrifugation to remove ribosomes. After removing nonspecific proteins 3 times, the serum albumin fraction was precipitated by adding carrier rat serum albumin and its antibody. The details of these procedures and the methods of radioactivity determination were described previously (2). The results are shown as radioactivity (dpm) per mg ribosomal RNA.

	free	loose	tight
nascent protein	10,800	6,640	13,800
serum albumin fraction	64	254	3,990
nonspecific proteins (3rd)	30	68	59

ribosomes in loose polysomal fraction is small, thus confirming the existence of loose ribosomes in liver cells.

In order to obtain evidence for functional differences

The amount of contaminated free ribosomes in the loose ribosome fraction which was calculated from ^{3}H-radioactivity of this fraction and the ratio of ^{14}C- to ^{3}H-radioactivity of free ribosomes, is shown as shadowed area.

between these two types of bound ribosomes, amounts of pulse-labeled nascent albumin on the ribosomes were measured by the immunological method.[2] Table 2 shows that serum albumin is exclusively synthesized on tight ribosomes and the portion of nascent albumin in the nascent proteins is as high as 30 %.

These results indicate that loose ribosomes and tight ribosomes in liver cells are functionally different from each other and secretary proteins may be exclusively synthesized on tight ribosomes. The functions of loose ribosomes are left to be clarified.

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