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Differences in Size, Structure and Function of Free and Membrane-Bound Polyribosomes of Rat Liver

EVIDENCE FOR A SINGLE CLASS OF MEMBRANE-BOUND POLYRIBOSOMES

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Free loosely bound and tightly bound polyribosomes were separated from rat liver homogenate by salt extraction followed by differential centrifugation, and several of their structural and functional properties were compared to resolve the existence of loosely bound polyribosomes and verify the specificity of the separation. The free and loosely bound polyribosomes have similar sedimentation profiles and polyribosome contents, their subunit proteins have similar electrophoretic patterns and their products of protein synthesis *in vitro* show a close correspondence in size and amounts synthesized. In contrast, the tightly bound polyribosomes have different properties from those of the free and loosely bound polyribosomes; their average size is significantly smaller; their polyribosome content is higher; their 60S-subunit proteins lack two components and contain four or more components not found elsewhere; their products of protein synthesis *in vitro* differ in size and amounts synthesized. These observations show that rat liver membranes entrap a large fraction of the free polyribosomes at low salt concentrations and that these polyribosomes are similar to those of the free-polyribosome fraction and are different from those of the tightly bound polyribosome fraction in size, structure and function.

Free polyribosomes are usually separated from membrane-bound polyribosomes by isopycnic centrifugation (Bloemendahl *et al.*, 1964; Webb *et al.*, 1964; Blobel & Potter, 1967). However, the completeness of this separation has not been verified. For this reason, studies demonstrating heterogeneous populations of membrane-associated polyribosomes, i.e. 'loosely' and 'tightly' bound (Rosbash & Penman, 1971*a*,*b*; Lee *et al.*, 1971; Bleiberg *et al.*, 1972; Tanaka & Ogata, 1972), have generally been difficult to interpret. It therefore seemed appropriate to compare comprehensively the structural and functional properties of free, loosely bound and tightly bound polyribosomes in order to ascertain whether any distinguishing features exist which could be used to identify the polyribosomes and verify the specificity of the separation.

Experimental

Materials

Chemicals and solutions. All of the chemicals used have been described elsewhere (Ramsey & Steele, 1976*a*). All solutions were made with glass-distilled water, filtered (0.45 μm pore size; Millipore Corp., Bedford, MA, U.S.A.) and stored at −20°C. HK(x)M medium contained 50mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH7.4 at 4°C, 25–500mM-KCl and 5mM-$MgCl_2$; (x) denotes the KCl concentration indicated in mM.

Animals. Male Sprague–Dawley rats (Biolab, Minneapolis, MN, U.S.A.), 140–180g, were maintained in constant environmental conditions for 3–5 days with free access to the Rockland Mouse/Rat Diet (Teklad, Winfield, IA, U.S.A.) and water, and then starved overnight (16h) and treated as described previously (Ramsey & Steele, 1976*a*).

Methods

Separation of free, loosely bound and tightly bound polyribosomes. Liver homogenate (25%, w/v) was prepared in 250mM-sucrose containing 3mM-GSH (reduced glutathione) and HK(75)M medium, and centrifuged consecutively for 2min at $740g_{max.}$ and 12min at $135000g_{max.}$ (SW27.1 rotor; Beckman) to separate free polyribosomes from the rough microsomal fraction (Venkatesan & Steele, 1972*b*). The supernatant containing free polyribosomes was decanted and stored at 0°C. The pellet was suspended by homogenization with 3vol. (w/v, based on liver wet wt.) of 250mM-sucrose containing 3mM-GSH and HK(250)M medium to release loosely bound poly-

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ribosomes, and the mixture was centrifuged as above to sediment the rough microsomal fraction. The supernatant containing loosely bound polyribosomes was decanted and stored at 0°C. The pellet was suspended in 50% cell sap/HK(250)M medium, treated with 1% Triton X-100, and centrifuged for 5min at $1470g_{max.}$ to remove nuclei as described previously (Ramsey & Steele, 1976*a*). The supernatant containing tightly bound polyribosomes was decanted, treated with 1.3% (w/v) deoxycholate, centrifuged for 10min at $27000g_{max.}$ (HB-4 rotor Sorvall) to remove deoxycholate-insoluble material, and stored at 0°C.

Isolation of polyribosomes. For determination of the effect of KCl concentration on polyribosome release (Table 1), the extract and pellet fractions obtained from the initial centrifugation of the homogenate were treated with Triton X-100 and deoxycholate and centrifuged for 10min at $27000g_{max.}$ to remove deoxycholate-insoluble material. Portions (5ml) of each supernatant were layered over 4ml of 1.38M-sucrose/HK(250)M medium and centrifuged for 4h at $226000g_{max.}$ (50Ti rotor, Beckman) to pellet the polyribosomes. The pellets were suspended in water for chemical analysis and occasionally in 0.5% (w/v) sodium dodecyl sulphate for A_{260}/A_{280} measurements; ratios (±S.E.M.) were 2.05 ± 0.01 and 2.07 ± 0.00 ($n = 12$) for free and bound polyribosomes respectively.

For determination of both polyribosome distribution and contamination with rough microsomal fraction in salt-releasable polyribosomes (Table 2), portions (3ml) of the free and loosely bound polyribosome fractions with and without detergent treatment as above, and of the tightly bound polyribosome fraction, were layered over discontinuous gradients composed of 3ml each of 1.38M- and 2.0M-sucrose/HK(250)M medium and centrifuged for 20h at $174000g_{max.}$ (50Ti or 65 rotor, Beckman). The RNA in the 2M-sucrose layer and pellet was determined as described previously (Ramsey & Steele, 1976*a*).

Sedimentation analysis of polyribosomes. The polyribosome fractions after treatment with detergents and low-speed centrifugation as above were divided into two portions; one portion was stored at 0°C (sample) and the other (3ml) was centrifuged for 75min at $226000g_{max.}$ (50Ti rotor) to obtain a ribosome-free blank. Equal portions of each were layered over identical 20–47% (w/w) linear sucrose gradients with an overlay (2ml) containing 18% sucrose, HK(250)M, 1% Triton X-100 and 1.3% deoxycholate, and centrifuged for 4.5h at $131000g_{max.}$ in opposite buckets of the rotor (SW27). The gradient solutions contained HK(250)M, 0.5mM-EDTA and charcoal-purified commercial sucrose. After centrifugation, each set of gradients was monitored simultaneously at 254nm with a dual-beam analyser (UA-5; ISCO, Lincoln, NB, U.S.A.) and the difference profile was recorded (J. C. Ramsey & W. J. Steele, unpublished work). The baseline across the polyribosome region of the gradient was flat when samples were treated with ribonuclease, indicating that the profiles were due to polyribosomes. The size distribution of polyribosomes was determined by planimetry of the absorbance profiles (Ramsey & Steele, 1976*a*). The assignments of sedimentation coefficients and polyribosome size classes were made by comparison with profiles obtained on isokinetic gradients (McCarty *et al.*, 1974).

Distribution of ^{14}C-labelled polyribosomes. [6-^{14}C]Orotate-labelled membrane-free polyribosomes were prepared in the presence of HK(250)M medium and used as described previously (Ramsey & Steele, 1976*a*), except that they were sonicated twice for 5s at 0°C (Branson model W-185-C, power supply at a setting of 1 with a micro-tip) after isolation to disperse aggregates arising from sedimentation.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of ribosomal subunits. Polyribosomes were isolated from the various fractions without previous treatment by centrifugation through discontinuous 1.38–2.0M-sucrose gradients containing HK(500)M, and then resuspended and converted into subunits by treatment with puromycin/high salt as described by Blobel & Sabatini (1971). The subunits were separated by centrifugation on 10–40% (w/w) linear sucrose gradients containing HK(500)M, collected and recovered by centrifugation for 18h at $193000g_{max.}$ (50.2Ti rotor; Beckman). The pellets were suspended in water, treated with ribonuclease A ($1.25\mu g/A_{260}$ unit), dissolved in sample buffer and subjected to electrophoresis (2 A_{260} units/gel) on 15% polyacrylamide gels (0.6cm × 22cm, with a 1cm 3% polyacrylamide stacking gel) at 2mA/gel for 15h as described by Laemmli (1970). After electrophoresis, the gels were stained with Coomassie Brillant Blue G-250, and destained with 7% (v/v) acetic acid as described by Diezel *et al.* (1972). Molecular-weight calibration of the gels was obtained by using ribonuclease (apparent mol.wt. 17000), deoxyribonuclease (31000) and bovine serum albumin (apparent mol.wt. 65000).

Product analysis of cell-free protein synthesis. Polyribosomes were isolated from the various fractions without previous treatment and assayed essentially as described previously (Ramsey & Steele, 1976*a*). Polyribosomal suspensions (6.8 A_{260} units/ml) were incubated for 30min with either [4,5-^3H]leucine (10nmol/ml, 2Ci/mmol) or [U-^{14}C]leucine (10nmol/ml, 312Ci/mol) in a complete cell-free system. After incubation, the samples were cooled to 0°C, and a portion from the loosely bound polyribosome system (^3H-labelled) was combined with an equal volume from the free or the tightly bound polyribosome system (^{14}C-labelled) and centrifuged for 2h at $200000g_{max.}$ (50Ti rotor) to remove

the ribosomes. Released polypeptides in the supernatants were precipitated with acid, washed, dissolved in sample buffer, and subjected to electrophoresis on 10% polyacrylamide gels (1.46cm × 12cm, with a 1cm 3% stacking gel) at 5mA/gel, as described by Laemmli (1970). After electrophoresis, the gels were sliced (1.8mm discs), digested in NCS solubilizer, acidified with acetic acid and counted for radioactivity as described previously (Ramsey & Steele, 1976*a*).

Chemical analysis. RNA was determined by the method of Fleck & Munro (1962), except that absorbance measurements were made in 0.2M-$HClO_4$/0.5% (w/v) sodium dodecyl sulphate to solubilize non-sedimentable material. DNA and protein were determined by the methods of Burton (1956) and Lowry *et al.* (1951), with calf thymus DNA and bovine serum albumin as standards respectively.

Results

Extraction of loosely bound polyribosomes from rat liver homogenate

Although the releasing effect of high KCl has been studied previously, most of the studies deal with rough microsomal fractions; hence it was important to ascertain whether the optimal conditions for extracting rough microsomal fraction were the same for whole liver homogenate. KCl promotes the release of polyribosomes that are not anchored to the membrane through nascent chains, i.e. 'loosely' bound (Adelman *et al.*, 1973). Table 1 shows that maximum release of loosely bound polyribosomes was obtained by raising the KCl concentration in the homogenate to about 250mM, and that the extent of release was roughly proportional to the KCl concentration. That the RNA is ribosomal is described elsewhere (Venkatesan & Steele, 1972*a*). The 25mM-KCl present in traditional polyribosome buffers extracted about one-third of the total salt-releasable polyribosomes. High KCl concentrations (>250mM) not only released loosely bound polyribosomes but also caused a change in the sedimentation rate of the rough microsomal fraction, thereby increasing the degree of rough-microsomal contamination in the extract. The detergent treatment used in the present study precludes an analysis of this contamination; however, as shown below, the contamination was minimal with moderate KCl concentrations. Thus,

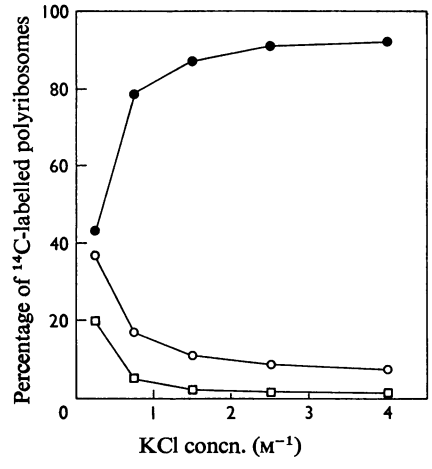

Fig. 1. *Effect of KCl concentration on the distribution of ^{14}C-labelled polyribosomes in free + loosely bound and membrane-associated polyribosome fractions*

[^{14}C]Orotate-labelled polyribosomes (specific radioactivity 2.5 × 10^5 d.p.m./mg of RNA) were added to homogenates (0.1mg of RNA/ml) prepared in media containing various KCl concentrations and the fractions prepared therefrom were assayed for radioactivity as described under 'Methods'. ●, Free + loosely bound fraction; ○, membrane-associated fraction; □, nuclear fraction.

Table 1. *Effect of KCl concentration on the distribution of homogenate RNA in free + loosely bound and membrane-associated polyribosomes isolated from 1.0g wet wt. of rat liver*

A 25% (w/v) homogenate of rat liver was prepared in 250mM-sucrose/HK(x)M medium containing 3mM-GSH and centrifuged; the free + loosely bound and membrane-associated polyribosomes were isolated as described under 'Methods'. Recovery was calculated on the basis of 100% for the total homogenate RNA. The values represent the averages of triplicate measurements from three experiments, which gave nearly identical results.

KCl (mM)	Total RNA (μg)	Free + loosely bound polyribosomes (μg)	Free + loosely bound polyribosomes (%)	Membrane-associated polyribosomes (μg)	Membrane-associated polyribosomes (%)	Total RNA recovery (%)	100 × Free + loosely bound/total polyribosome ratio
25	8990	822	9.1	5970	66.4	75.6	12.1
75	8650	1360	15.7	5210	60.2	75.9	20.7
150	8610	1950	22.6	4900	56.9	79.6	28.5
250	8920	2220	24.9	4720	52.9	77.8	32.0
400	8970	2263	25.2	4590	51.1	76.4	33.0

Table 2. *Distribution of homogenate RNA in free, loosely bound and tightly bound polyribosomes and rough-microsomal contamination in free and loosely bound polyribosomes*

A 25% (w/v) homogenate of rat liver was treated sequentially with 75mM- and 250mM-KCl media and centrifuged; the free and loosely bound polyribosome fractions with and without detergent treatment and the tightly bound polyribosome fraction were centrifuged on discontinuous sucrose gradients as described under 'Methods'. The 2.0M-sucrose layer and pellet from the gradients were analysed for RNA by chemical analysis. Recovery was calculated on the basis of 100% for the total homogenate RNA. The values represent the averages of triplicate measurements from two experiments, which gave nearly identical results.

	Percentage of homogenate RNA								
	Free		Loosely bound		Tightly bound		Total RNA recovery (%)	100 × Free/ total polyribosome ratio	100 × Free + loosely bound/total polyribosome ratio
Treatment	2M-sucrose layer	Pellet	2M-sucrose layer	Pellet	2M-sucrose layer	Pellet			
−Detergents	3.3	11.7	2.0	7.0	8.4	41.1	73.5	20.4	32.7
+Detergents	3.5	14.2	2.2	9.4	8.4	41.1	78.8	22.5	37.2

in subsequent experiments, 250mM-KCl was used to extract loosely bound polyribosomes.

To verify the specificity of the extraction and fractionation procedures, [^{14}C]orotate-labelled salt-releasable polyribosomes were added to the homogenates. Fig. 1 shows, in agreement with the previous results, that maximum recovery of salt-releasable polyribosomes was obtained with 250mM-KCl. With this medium, only 1 and 8% of the labelled polyribosomes sedimented in the nuclear and tightly bound polyribosome fraction respectively, indicating that this cell-fractionation scheme enables one to separate most of the salt-releasable polyribosomes from the rough microsomal fraction without using isopycnic centrifugation.

Characterization of free, loosely bound and tightly bound polyribosomes

To understand the significance of the loosely bound polyribosomes and to demonstrate further the specificity of the fractionation scheme, free, loosely bound and tightly bound polyribosomes were separated from rat liver homogenate as described under 'Methods' and analysed for: (*a*) cross-contamination, (*b*) polyribosome size, (*c*) ribosomal-subunit protein patterns and (*d*) size distribution of polypeptides synthesized *in vitro*.

Cross-contamination. Because we wished to compare the size of the free and loosely bound polyribosomes without previous purification, it was essential to determine the degree of rough-microsomal contamination in the two fractions. This was done by centrifuging equal amounts of the polyribosome supernatants with and without detergent treatment on discontinuous sucrose gradients. Table 2 shows that polyribosome recovery was about 15–17% higher in the deoxycholate-treated samples, indicating there was no significant difference in rough-microsomal contamination between the two fractions. The degree of contamination was identical whether the polyribosomes were extracted in a sequential manner as above or in single-step manner (results not shown), indicating that the sedimentation rate of the rough-

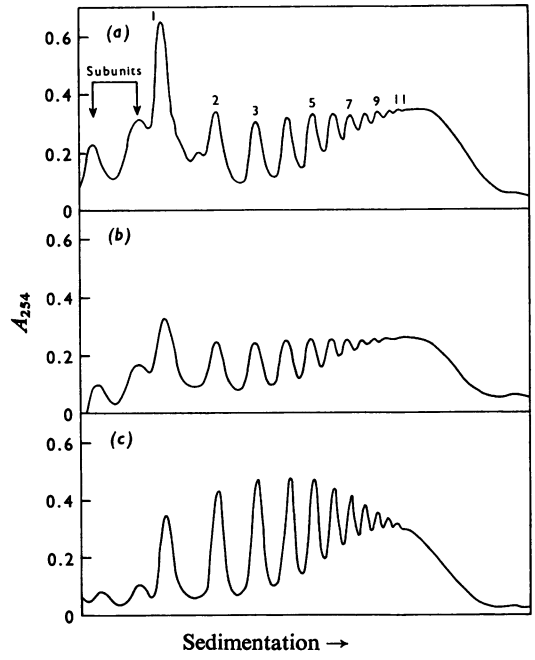

Fig. 2. *Sedimentation profiles of free (a) loosely bound (b) and tightly bound (c) polyribosome fractions from rat liver*

Polyribosome fractions were isolated from a whole homogenate of rat liver tissue, treated with detergents, centrifuged (1.2ml of the free and loosely bound and 0.5ml of the tightly bound fractions) along with their respective ribosome-free blanks and analysed as described under 'Methods'.

microsomal fraction was not altered by the sequential procedure. For comparisons other than size, the free and loosely bound polyribosomes were purified without detergent treatment, and hence contained no membrane-bound polyribosomes (Ramsey & Steele, 1976*a*). Combining the information in Table 2 with estimates of the salt-releasable polyribosome contamination in tightly bound polyribosomes (Fig. 1), we conclude that 33% of the total cytoplasmic polyribosomes are salt-releasable, and 67% are tightly bound to membranes.

Polyribosome size. This was investigated by using modified methods for sucrose-gradient analysis that enable one to visualize the entire complement of particles in each fraction and in the whole homogenate with the scheme used. Fig. 2 shows that the average size of the loosely bound polyribosomes was similar to that of the free polyribosomes, and was larger than that of the tightly bound polyribosomes. Identical results were obtained with portions of the same samples that had been stored at 0°C for 24h (results not shown); hence it is very unlikely that the smaller size of the tightly bound polyribosomes is due to differential ribonuclease activity. Quantification of the areas under the profiles showed that the proportion of large polyribosomes (aggregates with more than nine ribosomes) in the loosely bound fraction (47%) was similar to that in the free fraction (45%), and was greater than that in the tightly bound fraction (32%), in line with the above observation. The total polyribosome content of the loosely bound fraction (77%) was also similar to that of the free fraction (72%), and was lower than that of the tightly bound fraction (86%), as would be expected if 250mM-KCl leads to the release of particles that are not anchored to the membrane through nascent chains, i.e. subunits and possibly some monoribosomes. By both these criteria, loosely bound polyribosomes resemble free polyribosomes.

Ribosomal-subunit protein patterns. To ascertain whether the complement of subunit proteins might be different, subunits were prepared from polyribosomes isolated in the presence of 500mM-KCl and subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Fig. 3 (gels *a–c*) shows that there was no significant difference in the pattern of 40S-subunit proteins. In contrast, Fig. 3 (gels *d–f*) shows that the patterns of 60S-subunit proteins from the free and loosely bound polyribosomes were similar, and were different from the pattern for 60S-subunit proteins of the tightly bound polyribosomes in seven qualitatively significant components. The subunits from the free and loosely bound polyribosomes lacked bands 1, 2, 3, 5 and 7, and contained band 6 and a substantially greater amount of band 4 than subunits from the tightly bound polyribosomes. That these differences are not due to differential cross-contamination between subunit populations is

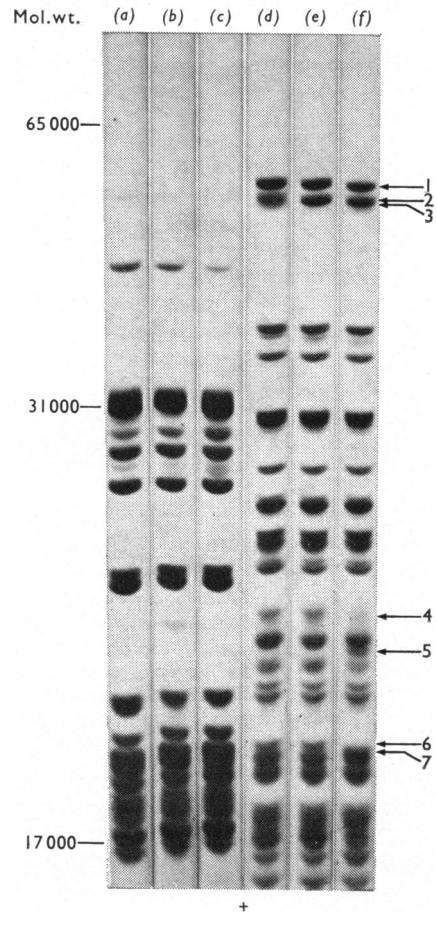

Fig. 3. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of 40S-subunit proteins from free (a), loosely bound (b) and tightly bound (c) polyribosomes and of 60S-subunit proteins from free (d), loosely bound (e) and tightly bound (f) polyribosomes*

Subunits were isolated from polyribosomes at various stages of the fractionation and subjected to electrophoresis (2 A_{260} units/gel) on 15% gels as described under 'Methods'. Arrows (1–7) indicate the positions of bands that have heterogeneous distributions.

shown by the absence of characteristic bands from the gel patterns; hence we conclude that the structural features of the 60S subunits of free and loosely bound polyribosomes are similar, and are different from those of the 60S subunits of tightly bound polyribosomes.

Polypeptides synthesized in vitro. To ascertain whether the functional properties of the polyribosomes might be different, polyribosomes from the loosely bound fraction and either the free or

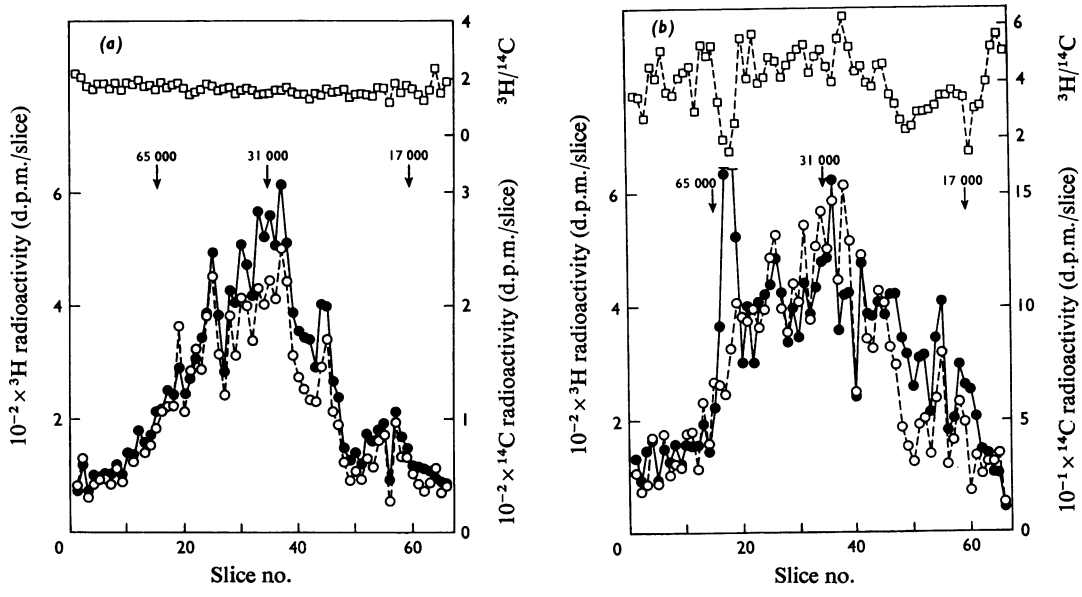

Fig. 4. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of polypeptides synthesized by free and loosely bound polyribosomes (a) and loosely bound and tightly bound polyribosomes (b) in vitro*

Free and tightly bound polyribosomes were incubated with [^{14}C]leucine, and loosely bound polyribosomes with [^{3}H]leucine, for 30min in a liver cell-free system and then the latter were combined separately with either free or tightly bound polyribosomes; the released polypeptides were isolated and subjected to electrophoresis (4mg of protein/gel) on 10% polyacrylamide gels and radioactivity was determined as described under 'Methods'. ●, Free or tightly bound polypeptides; ○, loosely bound polypeptides; □, ratio $^3H/^{14}C$.

tightly bound fraction were incubated with different isotopic forms of the same amino acid and then combined in pairs, and the released polypeptides were isolated and subjected to sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Fig. 4(*a*) shows that the molecular-weight distribution of polypeptides synthesized by the free polyribosomes was similar to that synthesized by the loosely bound polyribosomes. The $^3H/^{14}C$ ratio along the gel differed by less than 10% from the average (excluding ratios at the extreme ends of the gel), indicating that there were no large differences in the amounts and presumably types of polypeptides being synthesized by the two fractions. In contrast, Fig. 4(*b*) shows that the molecular-weight distribution of polypeptides synthesized by the loosely bound polyribosomes was strikingly different from that synthesized by the tightly bound polyribosomes. The $^3H/^{14}C$ ratio along the gel varied widely from the average, as would be expected if the two polyribosome populations are engaged in the synthesis of different sets of polypeptides. In this experiment 68, 54 and 44% of the total polypeptides were released by the free, loosely bound and tightly bound polyribosomes respectively; identical results were obtained when the extent of release was increased by increasing the ratio of cell sap to polyribosomes.

Discussion

The results show that rat liver membranes entrap a large fraction (up to 60%) of the free polyribosomes at low salt concentrations, and that these polyribosomes are similar to those of the free polyribosome fraction, and are different from those of the tightly bound polyribosome fraction in size, structure and function. Implicit in the results is the finding that the loosely bound polyribosomes are not a subclass of membrane-bound polyribosomes as has been previously believed (see the Introduction), but an artifact of cell fractionation. This finding confirms the suspicions of earlier workers about the origin and functional specialization of these polyribosomes (Tanaka & Ogata, 1972; Zauderer *et al.*, 1973; Nabeshima *et al.*, 1975) and agrees with the finding that the buoyant density of the loosely bound polyribosomes is similar to that of the free polyribosomes (Rosbash & Penman, 1971*a*). An important implication of this finding is that studies that have used 'low-salt' cell-fractionation schemes

in an attempt to show the functional specialization, distribution, heterogeneity or differential drug-sensitivity of free and bound polyribosomes should be re-evaluated, since it is now necessary to demonstrate that such schemes do not lead to incomplete separation of free polyribosomes from the rough-microsomal fraction.

The amount of polyribosome entrapment by membranes is roughly proportional to the salt concentration in the homogenate, and not to the force used in the initial centrifugation. This result agrees with the observations by Adelman *et al.* (1973) on rat liver rough-microsomal fractions; however, the extent of ribosome release as well as the optimal salt concentration and the amount of polyribosome breakdown were unusually high relative to that obtained here. These differences may reflect either the various incubation temperatures used, or more likely the activity of the ribonucleases and proteinases of the rough-microsomal preparations used.

Over 45% of the free and loosely bound polyribosomes sediment in the region of the gradient that corresponds to polyribosomes containing more than nine ribosomes. The loosely bound polyribosomes are therefore virtually identical in size with those in the free-polyribosome fraction. Quantification of the area under the profiles also showed that the total polyribosome content of the loosely bound fraction (79%) was comparable with that of the free fraction (77%). Thus there is no evidence for a differential distribution of mRNA populations in the loosely bound fraction, a conclusion consistent with earlier findings (Zauderer *et al.*, 1973), although our studies do not rule out the existence of minor differences. On the other hand, the small proportion of large polyribosomes (32%) in the tightly bound fraction from starved rats suggests that on average the bound polyribosomes synthesize somewhat smaller polypeptides than do the free polyribosomes. This suggestion gains support from the finding that the average size of the polypeptides synthesized by bound polyribosomes in rat liver is smaller than those synthesized by the free polyribosomes (J. C. Ramsey & W. J. Steele, unpublished work), and that starvation for up to 64h does not change the distribution between free and bound polyribosomes in rat liver (Ramsey & Steele, 1976*b*). Until now, no direct method has been available for observing the entire complement of free and bound ribosomes in rat liver. The difference profile procedure used here to analyse both polyribosome size and content is also of potential use in the quantification of both ribosome concentration and distribution as well as in the analysis of possible regulatory processes at the level of translation (J. C. Ramsey & W. J. Steele, unpublished work).

The patterns of 40S and 60S ribosomal-subunit proteins of the loosely bound polyribosomes on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis are identical with those of the 40S- and 60S-subunit proteins of free polyribosomes. Thus there is no evidence for a preferential localization of proteins in the loosely bound polyribosomes, which might function in their attachment to membrane. On the other hand, the 60S subunits from tightly bound polyribosomes lack two components present in ribosomal subunits of free (and loosely bound) polyribosomes and contain four or more components not found elsewhere. In previous studies there has been some disagreement about the existence of heterogeneous ribosomes in rat liver. Borgese *et al.* (1973) found that free 60S subunits contained one extra protein, whereas Hanna *et al.* (1973) and Hoffmann & Ilan (1977) observed no differences. This discrepancy may reflect either the sensitivity of the various electrophoretic separations, or more likely the presence of free-polyribosome contamination in the bound-polyribosome preparations used. The major difference in the present study is that the degree of cross-contamination in the bound polyribosomes is extremely low.

The role of ribosome heterogeneity in the operation of the subunit cycle in eukaryotic cells is unclear. Blobel & Dobberstein (1975) have proposed that ribosome binding to the membrane proceeds through an initial dissociation of one or more 60S-subunit proteins, thereby uncovering binding sites that recruit and bind several membrane receptor proteins to the ribosome. The similar pattern of heterogeneity observed in the present study appears to lend credence to such a model. However, since our results are based solely on electrophoretic mobility, we cannot exclude the possibility that observed differences are due to protein modification rather than exchange. The method used has, however, been shown to be relatively insensitive to minor protein modifications, i.e. phosphorylation and acetylation (Kabat, 1970; Liew & Gornall, 1973; Eil & Wool, 1973).

We have previously shown that the molecular-weight profile after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the polypeptides synthesized by free polyribosomes is different from that synthesized by bound polyribosomes (Ramsey & Steele, 1976*a*). The present data extend these studies to a population of bound polyribosomes in which free polyribosomes constitute no more than 5% of the total polyribosomes. Although studies of specific proteins are necessary, we can conclude from the data presented that the size-fractionation procedure described is reasonably specific and quantitative. Moreover, in contrast with procedures based on rate-zonal or isopycnic centrifugation, the size-fractionation approach is rapid and should therefore facilitate the isolation of undegraded free and bound polyribosomes from tissues with much ribonuclease activity like liver. From the data in Fig. 1 and Table 2, we find that approx. 33% of the cytoplasmic

ribosomes are free (salt-releasable) and 67% are bound to membranes. The ratio of free to bound ribosomes in rat liver is therefore 1:2 rather than 1:3 as reported previously (Blobel & Potter, 1967; Ramsey & Steele, 1976*a*).

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