Segregation of the Polypeptide Translocation Apparatus to Regions of the Endoplasmic Reticulum Containing Ribophorins and Ribosomes. I. Functional Tests on Rat Liver Microsomal Subfractions

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ABSTRACT A preparation of rat liver microsomes containing 70% of the total cellular endoplasmic reticulum (ER) membranes was subfractionated by isopycnic density centrifugation. Twelve subfractions of different ribosome content ranging in density from 1.06 to 1.29 were obtained and analyzed with respect to marker enzymes, RNA, and protein content, as well as the capacity of these membranes to bind 80S ribosomes in vitro. After removal of native polysomes from these microsomal subfractions by puromycin in a buffer of high ionic strength their capacity to rebind 80S ribosomes approached levels found in the corresponding native membranes before ribosome stripping. This indicates that in vitro rebinding of ribosomes occurs to the same sites occupied in the cell by membrane-bound polysomes. Microsomes in the microsomal subfractions were also tested for their capacity to effect the translocation of nascent secretory proteins into the microsomal lumen utilizing a rabbit reticulocyte translation system programmed with mRNA coding for the precursor of human placental lactogen. Membranes from microsomes with the higher isopycnic density and a high ribosome content showed the highest translocation activity, whereas membranes derived from smooth microsomes had only a very low translocation activity. These results indicate that the membranes of the rough and smooth portions of the endoplasmic reticulum are functionally differentiated so that sites for ribosome binding and the translocation of nascent polypeptides are segregated to the rough domain of the organelle.

The transfer of secretory and organellar proteins into the lumen of the endoplasmic reticulum (ER)[1] and the co-translational insertion of proteins into its membranes require the binding of polysomes to specific receptor sites on the cytoplasmic surface of the ER (34, 35). It is presently thought that this association is initiated by the interaction of a discrete polypeptide segment in the nascent chain (signal peptide) with a ribonucleoprotein particle termed the signal recognition particle (42–44) which guides the complex to a receptor in the ER membranes (14, 15, 30, 31). A strong ionic bond between the large ribosomal subunit and specific proteins in the membrane (1, 11, 34) may then be established to ensure the direct insertion into the membrane of polypeptide segments following the signal peptide. Furthermore, microsomal components must be involved in co-translational modification of the growing nascent chain, which include removal of the signal peptide (18) and transfer of asparagine-linked high mannose oligosaccharides (12, 17, 24). Aside from the signal recognition particle receptor, which in dog pancreas microsomes is a protein of M_r 72,000, it has been proposed that two transmembrane glycoproteins (ribophorin I and II) (20–23) and a protein of M_r 83,000 (38) are associated with the translocation apparatus, but specific functions of these proteins have not yet been established.

We have measured the ribosome-binding capacity and

[1] *Abbreviations used in this paper:* ER, endoplasmic reticulum; HPL, human placental lactogen; RM, rough microsomes.

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 DECEMBER 1984 2247-2253

translocation activity of ER membranes in rat liver microsomal subfractions prepared by density gradient centrifugation from a microsomal fraction containing ~70% of the total activity of ER marker enzymes in rat hepatocytes. Total liver microsomes were chosen for these studies because they yielded a spectrum of membrane vesicles derived from the ER covering a broad range of ribosomal content and microsomal subfractions separated by zonal centrifugation have been extensively characterized with respect to their biochemical composition and enzymatic activities (2–4, 7). The high recovery of membranes allowed us to determine if these functions are distributed throughout the ER as previously reported (8), or if they are confined to rough portions of this organelle.

The results reported here demonstrate that the ribosome-binding capacity of stripped microsomal subfractions approached the ribosome content of the corresponding native microsomes. Furthermore, the in vitro translocation and signal peptide processing activity was higher in membranes derived from subfractions with the greater ribosome load. This indicates that the rough and smooth portions of the ER represent two structurally and functionally distinct membrane domains.

MATERIALS AND METHODS

Materials: [^{35}S]Methionine (1,200 Ci/mmol), [^{3}H]uridine (25 Ci/mmol), Protosol, and Liquifluor were purchased from New England Nuclear (Boston, MA). Streptococcal nuclease and calf liver tRNA were from Boehringer Mannheim Federal Republic of Germany. Anti-human placental lactogen IgG, trypsin, and chymotrypsin were obtained from Miles Biochemicals, Inc. (Elkhart, IN). Ribonuclease inhibitor was purified from term human placenta as described (9). X-ray films (XAR-5) were from Kodak (Rochester, NY). All other chemicals were reagent grade and purchased from Sigma Chemical Co. (St. Louis, MO) or from Fisher Scientific Co. (Pittsburgh, PA).

Preparation of Microsomes and Microsomal Subfractions Female Wistar rats weighing 150–200 g were starved for 18 h before sacrificing by decapitation. Livers were removed, homogenized in ice-cold 250 mM sucrose buffered with 3 mM Imidazole-HCl pH 7.4, and fractionated by differential centrifugation into nuclear, large granular, microsomal and final supernatant fractions (3). Microsomes were further resolved into 12–13 subfractions by equilibrium centrifugation in a preformed sucrose gradient according to Beaufay et al. (7). The density distribution histograms of microsomal constituents were constructed as described elsewhere (5).

When used in the ribosome binding experiments, microsomes and microsomal subfractions were stripped of ribosomes by incubation in high salt buffer (500 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5) containing 1 mM puromycin, essentially as described by Adelman et al. (1). After incubation (30 min at 4°C and 15 min at 22°C), stripped microsomal membranes were recovered by sedimentation (20 min, 35,000 rpm, SW56 Beckman rotor, Beckman Instruments, Palo Alto, CA). After resuspension and washing in high salt buffer, the final sample was resuspended in binding buffer (100 mM KCl, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5).

Since microsomes stripped with puromycin inhibit in vitro translation, ribosomes were removed using chelating agents. 1-ml aliquots of the subfractions were used intact or after ribosome stripping which was carried out by adding to each aliquot a ten-fold volume of homogenization buffer containing 15 mM sodium-pyrophosphate. After incubation for 30 min at 4°C under continuous stirring, the suspension was added dropwise to an equal volume of homogenization buffer containing 700 mM KCl. Stripped microsomes were recovered by centrifugation (2 h, 35,000 rpm, Ti60 Beckman rotor, Beckman Instruments) in a sucrose step gradient (2 ml 0.6 M sucrose; 1 ml 1.8 M sucrose) buffered with 20 mM HEPES-KOH pH 7.6. The stripped membranes at the 0.6 M/1.8 M interphase were aspirated and resuspended in 1 ml of homogenization buffer using a Dounce homogenizer (tight pestle). To digest endogenous mRNA, 50 μl of 100 mM $CaCl_2$ and 10 μl (150 U) of streptococcal nuclease were added to each sample. After a 20-min incubation at 20°C the activation of the nuclease was stopped by adding 100 μl of 100 mM EGTA.

Determination of Reference Constituents: NADPH cytochrome *c* reductase, NADH cytochrome *c* reductase, glucose-6-phosphatase, esterase, and phospholipid were assayed as previously described (6). Data are presented in the form of frequency histograms as previously described (5). Protein was measured according to Lowry et al. (26). BSA was used as a standard.

In Vitro Binding of [^{3}H]Ribosomes to Microsomal Membranes: In vitro ^{3}H-ribosome-binding assays were carried out essentially according to Borgese et al. (11). For details see also Kreibich et al. (22). If not stated otherwise, the incubation mixtures contained 100 μg of membrane protein and 50 μg of ^{3}H-ribosomes in 0.12-ml binding buffer. Tritium-labeled 80S ribosomes used in this assay were prepared from HPC and 456 myeloma cells labeled for 2 d with [^{3}H]uridine as previously described (22). RNA determination was made as described by Fleck and Munro (13). Ribosome concentration was determined in 1% SDS using $E_{260} = 135$ (40) and a molecular weight of 4.5×10^6 (16). The RNA concentration was derived assuming that ribosomes contain 52% RNA. Specific radioactivity of the ^{3}H-ribosomes is given in the legends of the tables and figures. The in vitro ribosome-binding data are presented as Scatchard plots from which the apparent affinity constants and the number of ribosome binding sites were derived for each subfraction.

Preparation of Placental RNA: Total placental RNA was extracted from human term placenta as previously described (25, 41). In vitro translation of this RNA preparation followed by immuneprecipitation with anit-human placental lactogen (HPL) antibodies demonstrated that pre-human placental lactogen (pre-HPL) was the major translation product.

Assay for Translocation Capacity of Microsomal Subfractions: Each microsomal subfraction (2.5 μg of membrane protein, subtracting protein contributed by membrane bound ribosomes) was added to an in vitro translation system consisting of a nuclease-treated rabbit reticulocyte lysate (32) programmed with mRNA extracted from human term placenta. The final K^+ and Mg^{++} concentrations were adjusted to 90 and 1.2 mM, respectively, by addition of the acetate salts. The 25-μl translation mixture also contained placental RNAse inhibitor (37), 2.5 μg of calf liver tRNA. and 12.5 μCi of [^{35}S]methionine. After 1.5 h of incubation at 27°C, the samples were cooled to 4°C. To one-half of the translation mixture, trypsin and chymotrypsin (20 μg/ml each) were added and incubated at 4°C for 30 min. Aliquots (12.5 μl) of each translation mixture were analyzed by SDS PAGE (12% polyacrylamide; 29) and the dried gels were exposed to X-ray films.

In vitro translated pre-HPL and proteolytically processed HPL were identified by immuneprecipitation using rabbit anti-HPL IgG. To determine the efficiency of processing of pre-HPL to HPL the respective labeled bands were excised from the dried gels, solubilized with Protosol, and [^{35}S]radioactivity was determined by scintillation counting.

Since pre-HPL contains seven methionine residues while HPL only six, percent processing was determined as follows: Percent processing = counts per minute in HPL × 1.17 × 100/cpm in HPL × 1.17 + counts per minute in pre-HPL.

Electron Microscopy: Rough microsomes (RM) were resuspended in binding buffer (3.5 mg protein/ml) and 100 μl was kept as a control (sample A). The remaining suspension of RM was stripped with high salt buffer containing 1 mM puromycin. The stripped membranes were resuspended in binding buffer and an aliquot (sample B) was saved. Two samples (150 μg each) of stripped membranes were incubated with 80S ribosomes (50 μg each) and in vitro ribosome binding was performed as described above. The top fractions were pooled and diluted with Imidazole buffer (3 mM, sample C). Samples A–C were sedimented (5 min, 135,000 *g* Beckman airfuge rotor). The pellets were resuspended in Imidazole buffer and glutaraldehyde was added (1% final). Sediments obtained after 60 min of fixation (4°C) were rinsed, postfixed with OsO_4, stained en bloc with uranyl acetate, and embedded in Epon. Thin sections were poststained with lead acetate.

RESULTS

Characterization of Microsomes and Microsomal Subfractions

For total microsomes utilized in the studies reported in this and the following paper (27), almost 70% of the total cellular ER membranes were recovered as indicated by the recovery of characteristic ER enzymes (Table I) such as NADPH cytochrome *c* reductase, glucose-6-phosphatase, and esterase. This corresponds to ~33, 11.7, and 4.4 mg of protein, phospholipid, and RNA per gram of liver, respectively.

The different ER constituents were, however, heterogeneously distributed in the subfractions obtained by isopycnic centrifugation (3, 4, 7) (Fig. 1). Subfractions with a high ribosome content were poorer in NADPH and NADH cyto-

TABLE I

Biochemical Characterization of Liver Homogenates and of Total Microsomes

Constituent	Content in liver homogenates	Liver content in microsomes*	No. of experiments
	mg/g liver	*%*	
Protein	204.4 ± 5.9	16.15 ± 0.49	2
Phospholipid	29.6	39.4	1
RNA	7.60 ± 0.08	57.6 ± 2.7	3
	U/g		
NADPH cytochrome c reductase	3.93 ± 0.81	66.7 ± 1.1	4
NADH cytochrome c reductase	107.3	67.1	1
Glucose-6-phosphatase	19.6 ± 4.1	67.2 ± 5.4	4
Esterase	267 ± 92	69.7 ± 5.2	2

* Liver content is defined as the sum of the amounts recovered in the nuclear fraction, large granules, microsomes and final supernate. The recoveries were 82–102%.
Values are means ± maximal deviations.

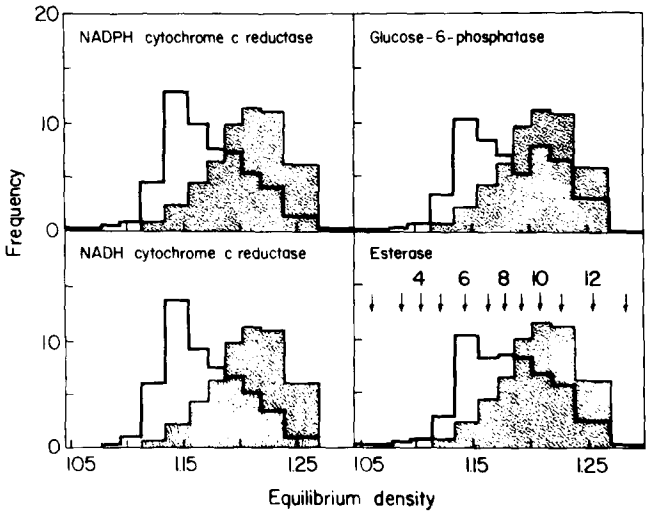

FIGURE 1 Distribution of rat liver microsomal constituents in subfractions of different density. Total microsomes were subfractionated by isopycnic centrifugation in the E-40 rotor (5, 7) containing a linear sucrose gradient buffered with 3 mM Imidazole-HCl (pH 7.4). The activities of NADPH cytochrome c reductase, NADH cytochrome c reductase, glucose-6-phosphatase, and esterase, as well as RNA content (shaded areas), were determined in each subfraction. In the lower right, fraction numbers are indicated by arrows.

chrome *c* reductases than smooth microsomes; whereas the RNA peak corresponded to a density of 1.22, the flavoprotein content was highest at a density of 1.14. As previously presented (7) other microsomal constituents, including total protein, phospholipid (not shown), esterase, and glucose-6-phosphatase, were more evenly distributed throughout the gradient, with peaks or shoulders at both high and low densities.

Ribosome-binding Capacity of Total Microsomes and Microsomal Subfractions

It has previously been shown that specific sites on rough microsomal membranes, which are exposed only after the membranes are stripped of native ribosomes, can rebind inactive 80S ribosomes in media of physiological ionic strength (11, 34). We compared the varying ribosome loads of the microsomal subfractions (see Fig. 1) with the capacity of the respective membranes to bind ribosomes in vitro after native polysomes were removed by treatment with puromycin in a high salt buffer.

As shown in Table II and the *inset* in Fig. 2 there was a remarkable agreement between the ribosome-binding capacity (107 μg of ribosomal RNA per mg of membrane protein at saturation; see Table II) of stripped membranes obtained from total microsomes and the ribosome content of the native microsomes (97 μg of ribosomal RNA per milligram membrane protein). It should be noted that even though microsomal subfractions derived from the total microsomes varied widely in ribosome-binding capacity (Table II and Fig. 2), similar apparent affinity constants could be calculated from the different Scatchard plots (Table II). The total amount of ribosomes rebound to subfractions 5–12 was calculated to be 21.3 mg of RNA, which represents 83% of native RNA content.

The values in Table II were used to compute a density distribution profile of the in vitro ribosome-binding capacity of the different membranes that in Fig. 3 is shown together with the distribution of RNA in the native subfractions. The similarity of the two profiles suggests that 80S ribosomes bind in vitro to the same sites that are occupied by membrane bound ribosomes in vivo. Smooth microsomal subfractions (below density = 1.14), which contain mainly vesicles derived from the smooth ER (4, 7), had very little, if any, ribosome-binding capacity. Since the results in Fig. 3 are represented as frequency distributions, an even better correlation between ribosome binding in vitro and RNA content of the subfractions would have been obtained if subfraction 12 did not have such an unexpectedly low ribosome-binding capacity. The low binding capacity of this densest subfraction was consistently observed but is unexplained since it contains rather high levels of ribophorins (27). Since this fraction contained <5% of the total glucose-6-phosphatase activity of the total microsomes it was not considered in the linear regression analysis of in vitro ribosome binding and ribosome content shown in Fig. 4 which gave a correlation coefficient of 0.98. This value indicates that the number of unoccupied binding sites in native subfractions is low. This was confirmed by in vitro ribosome-binding experiments using native (nonstripped) microsomal subfractions. In all cases the ribosome-binding capacity of the microsomal subfractions was very low ranging from 10–25 μg ribosome/mg of microsomal membrane. Significant binding was detected only after removal of native ribosomes.

Electron microscopy (Fig. 5) showed that at saturation, stripped vesicles (Fig. 5 *B*) derived from a heavy rough microsomal subfraction (Fig. 5 *A*) acquired a ribosome load (Fig. 5 *C*) comparable to that of the native membranes. It was also apparent that ribosomes did not form aggregates; trapping of unbound ribosomes was insignificant, and binding occurred directly to membrane surfaces.

Translocation Capacity of Microsomal Subfractions

The activity of microsomal subfractions in co-translational translocation of nascent polypeptides was determined in an in vitro translation system programmed with placental RNA. In the absence of microsomal membranes the major ^{35}S-labeled translation product of human placental RNA is pre-

TABLE II

Binding of ^{3}H-Ribosomes to Microsomal Subfractions Obtained by Density Gradient Centrifugation

Subfraction number‡	Density	A_{260}/A_{280}* Before stripping	A_{260}/A_{280}* After stripping	RNA§	[^{3}H]RNA bound‖		K_a¶
	g/cm			%	μg/mg protein**	μg/mg protein‡‡	$10^8\ M^{-1}$
5	1.142	1.10	0.78	4.4	26	13	1.04
6	1.154	1.30	0.77	5.4	37	24	2.12
7	1.166	1.37	0.80	7.2	58	40	1.94
8	1.178	1.54	0.83	10.1	83	58	2.16
9	1.193	1.71	1.06	16.0	130	101	1.36
10	1.212	1.78	1.12	20.4	171	195	0.81
11	1.236	1.80	1.14	19.0	201	227	0.92
12	1.256	1.95	1.02	11.4	236	72	2.31
Total Microsomes		1.34	0.79	100	93	107	1.73

Total microsomes and microsomal subfractions (5 to 12) were stripped of ribosomes with puromycin (1 mM) in a buffer of high ionic strength (500 mM KCl, 50 mM Tris-HCl, pH 7.2; 5 mM $MgCl_2$). After suspension in a buffer of physiological ionic strength, aliquots containing the same amounts of stripped membranes were incubated with increasing concentrations of ^{3}H-labeled ribosomes. Ribosomes bound to microsomal membranes were separated from unbound ribosomes by floatation of the membranes in a sucrose density gradient essentially as described (11, 22). The incubation mixture contained in a final volume of 120 μl, 100 μg of stripped membrane protein and increasing amounts of ^{3}H-ribosomes. The actual amounts of ribosomes added were calculated from the total amounts recovered in the gradient taking into consideration the specific activity of the ^{3}H-ribosomes (2,690 cpm/μg ^{3}H-ribosomes).

‡ Microsomal subfractions 5–12 contained 87, 82, and 94% of the total glucose-6-phosphatase activity, protein, and RNA content, respectively.

* The efficiency of the stripping procedure was monitored by comparing the A_{260}/A_{280} ratios before and after removal of bound ribosomes.

§ The total amounts of microsomal RNA and protein loaded on the gradient were 29.8 and 322 mg, respectively. The recoveries for RNA and protein in the gradient subfraction were 91 and 98%, respectively.

‖ Calculated from the intercepts with the abscissa on Scatchard plots shown in Fig. 2.

¶ The apparent affinity constant was calculated from the slope of the Scatchard plots shown in Fig. 2.

** Unstripped, native microsomal membranes.

‡‡ Stripped microsomal membranes.

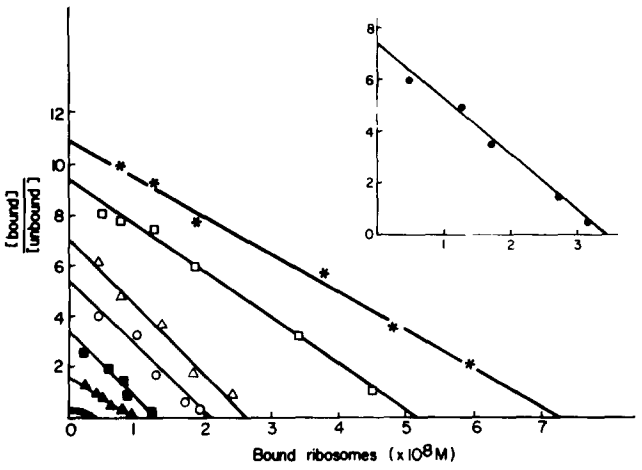

FIGURE 2 Scatchard plots of in vitro ribosome binding to total microsomes and microsomal subfractions. Microsomal subfractions and total microsomes (*inset*), each stripped of ribosomes, were incubated with increasing amounts of ^{3}H-labeled ribosomes. The results are represented in the form of Scatchard plots. The position of each line was established by the least square method. Apparent affinity constants for ribosome binding and the number of binding sites were calculated from the slope of each line and from its intercept with the abscissa, respectively (see Table II). Subfractions: 5, ●; 6, ▲; 7, ■; 8, ○; 9, △; 10, □; 11, * (for subfraction numbers see lower right of Fig. 1). Subfraction 12, which gave an unexpectedly low values, is not represented.

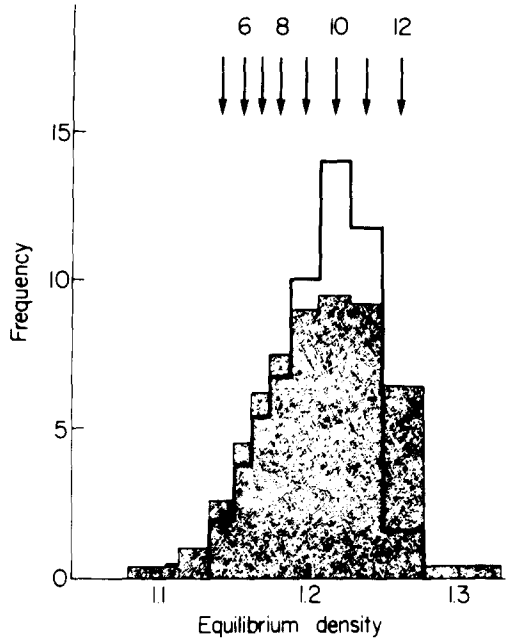

FIGURE 3 Comparison of the density distribution of the in vitro ribosome-binding capacity of stripped microsomal subfractions with the RNA content of the corresponding native subfractions. The density distribution of in vitro rebound ^{3}H-ribosomes (solid line) was computed with the data given in Table II. The distribution of RNA (shaded area) is redrawn from Fig. 1. Fraction numbers are indicated by arrows.

HPL (M_r 21,500), whereas co-translational processing yields HPL (M_r 19,000) (39). Microsomal subfractions with a high RNA content (subfractions *8–11*) were very efficient in translocation and proteolytic processing of nascent pre-HPL (Fig. 6, *B–D*). The polypeptides sequestered in the lumen of such vesicles were therefore inaccessible to the added protease which rapidly degraded pre-HPL representing ~50% of the total translation products. On the other hand, subfractions that contained significant amounts of NADPH cytochrome *c* reductase (fractions *4–6* in Fig. 6 *A*) but had a low RNA content, and therefore are largely derived from smooth portions of the ER, were almost devoid of translocation and processing activity.

A plot of the relative translocation activity of the microsomal subfractions is presented in Fig. 6 *D* together with the

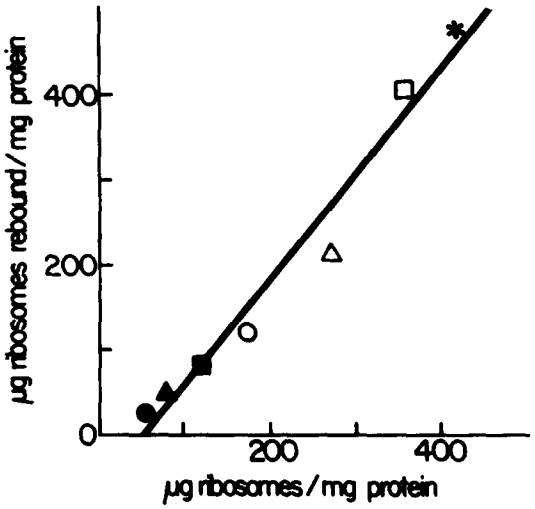

FIGURE 4 Correlation between the ribosome-binding capacity of stripped microsomal subfractions and the ribosome content of the corresponding native subfractions. The values for saturation binding of ^{3}H-80S ribosomes of subfractions 5–11, as well as the RNA to protein ratios of the corresponding native microsomes, are those from Table II. The regression line disregards the values for fraction 12 which showed consistently lower ribosome rebinding than expected from its RNA content before ribosome stripping. The correlation coefficient is 0.98.

corresponding RNA/protein ratios. A striking positive correlation is apparent. Table III shows however that the stripping procedure, which removes >70% of the RNA (4), did not significantly increase the co-translational translocation and processing capacity of the vesicles.

DISCUSSION

The results reported here demonstrate that practically all sites that in rough ER membranes are engaged in the co-translational insertion of nascent polypeptides in vivo, remain capable of rebinding 80S ribosomes after ribosome stripping. The high yield of ribosome rebinding achieved in this work with stripped rat liver microsomes is likely to reflect the gentle conditions of centrifugation in the zonal rotor which allow a fast subfractionation under low hydrostatic pressure (5), and therefore may prevent denaturation of protein components of the ribosomal binding sites. Subfractionation by isopycnic centrifugation resolves microsomes into a complete spectrum of vesicles, ranging from some bearing no ribosomes to others heavily loaded with bound polysomes (45). In every case levels of ribosome rebinding reflected the native ribosome content.

A quantitative interpretation of the tissue fractionation results and the related ribosome-binding experiments presented here is possible since a balance sheet was established that showed that the total number of binding sites recovered in the microsomal subfractions (83%) approaches that present in the original total microsomes. However, our results do not conclusively demonstrate that the constituents of ribosome-binding sites are absent from the smooth ER membranes. Conceivably, they may assemble into functional units only when newly initiated ribosomes attach to the ER membrane as has been proposed earlier (10). Such a model, however, would be compatible with our findings, only if it were assumed that once assembled, ribosome-binding sites do not disassemble when microsomes are stripped in vitro. In such a case

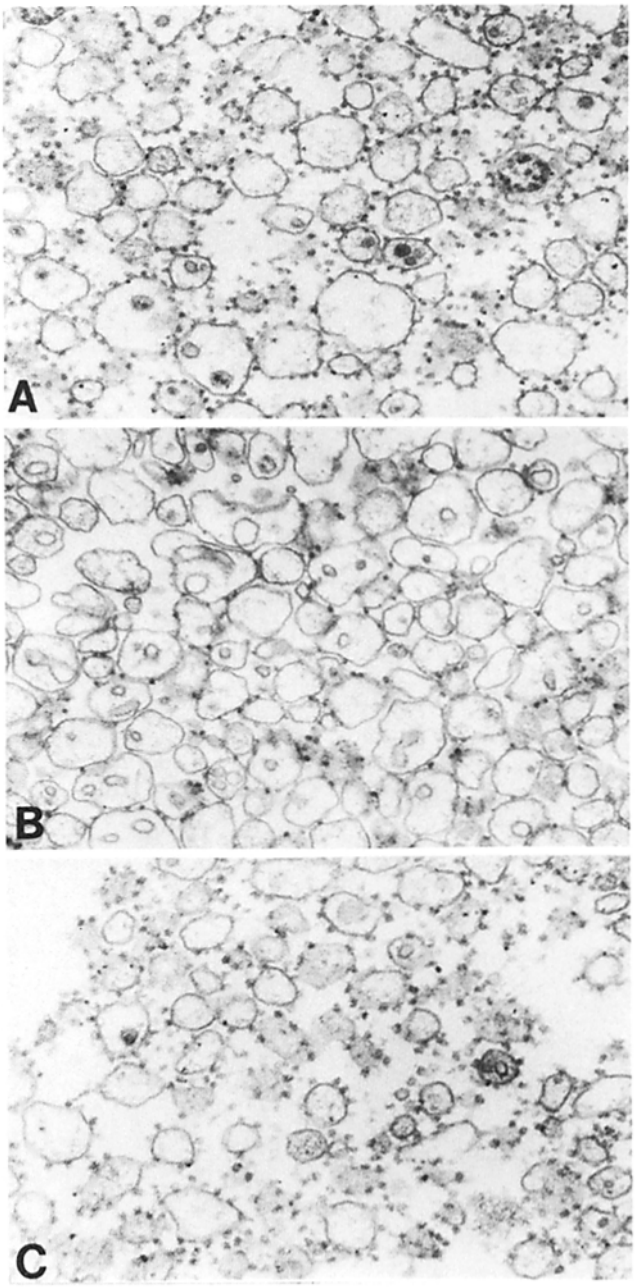

FIGURE 5 Thin section micrographs of native RM, RM stripped of ribosomes, and stripped RM after rebinding of 80S ribosomes in vitro. (*A*) Native RM (subfraction 10). (*B*) RM stripped of ribosomes with puromycin in HSB. A few ribosomes remain attached to the microsomal vesicles after the puromycin-high salt treatment. (C) Stripped RM after in vitro binding of inactive 80S ribosomes. × 75,000.

ribosome binding in vitro would reflect only the presence of preassembled sites. The experiments to determine the distribution of translocation sites for nascent chains in microsomal subfractions confirm and extend previous observations using RM and smooth microsomes prepared from rat liver or HeLa cells (19) and argue against the presence, in the smooth domain of the ER, of a pool of components of the translocation apparatus which could be recruited into functional units by nascent polysomes carrying chains with signal sequences. Our conclusions do not therefore support a view (8, 33) in which rough and smooth portions of the endoplasmic reticu-

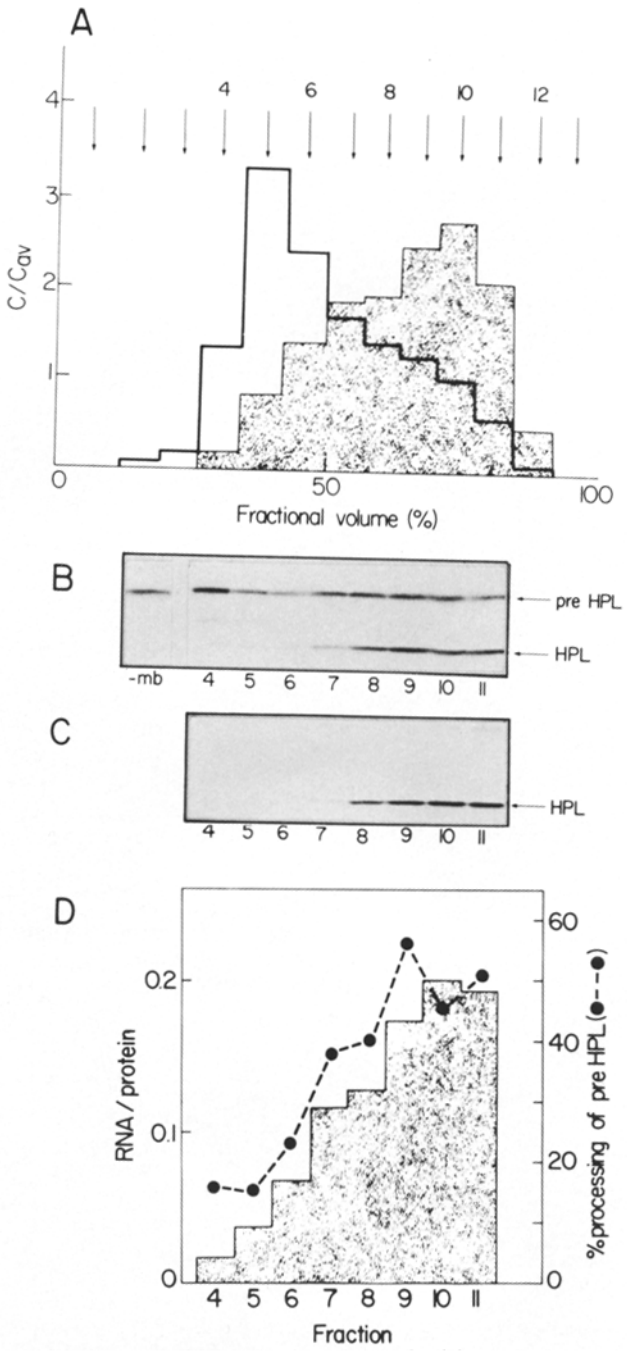

FIGURE 6 Activity of unstripped microsomal subfractions to translocate and process pre-HPL. Microsomal subfractions, ranging in density from 1.108 (fraction *4*) to 1.237 (fraction *11*) (see Table III) were obtained by isopycnic centrifugation in a sucrose gradient buffered with 3 mM Imidazole. The distribution of NADPH cytochrome *c* reductase (solid line) and RNA (shaded area) throughout the gradient are represented in *A*. The unstripped subfractions were added co-translationally to a rabbit reticulocyte lysate programmed with human term placental RNA (*B* and *C*). As a control, microsomes were omitted from the translation mixture (*-mb*). The experiment shown in *C* is a duplicate experiment of that depicted in *B* except that proteases were added posttranslationally (for details see Materials and Methods) demonstrating that HPL was translocated into the microsomal lumen and therefore protected. Aliquots of the translation mixture were analyzed by SDS PAGE (12%) and the dried gels were exposed to x-ray films. Only the middle section of the gels, where pre-HPL and HPL migrate, are shown. In *D*, the efficiency of microsomal subfractions to translocate and process pre-HPL is compared with their specific RNA content.

TABLE III

Translocation and Processing Activity of Microsomal Subfractions before and after Stripping of Ribosomes

Subfraction number*	Density	RNA: Protein	Percent processing‡	
			Native subfractions	Subfractions stripped of ribosomes
	g/cm^{-3}	$\mu g/mg$		
5	1.134	22	8	5
6	1.153	52	8	8
7	1.167	74	14	12
8	1.180	121	12	17
9	1.194	190	21	33
10	1.208	196	27	36
11	1.229	227	34	31
12	1.247	226	37	38

Microsomal subfractions were added either directly or after stripping of ribosomes (see Materials and Methods) to an in vitro rabbit reticulocyte translation system programmed with total RNA from human term placenta. After 90 min of incubation at 27°C, the [^{35}S]methionine-labeled translation products were separated by SDS PAGE. The dried gels were exposed to X-ray films and the [^{35}S]methionine-labeled pre-HPL and HPL bands were excised and the radioactivity measured. Areas of the gels of similar size but that had no apparent labeled band were excised and their radioactivity was measured and used as the background value. Percent processing was calculated as described in Materials and Methods.

* Microsomal subfractions 5–12 are similar to those described in Table II.

‡ The average incorporation of [^{35}S]methionine into HPL and pre-HPL was 15,000 cpm and was constant within 15% when different subfractions were assayed. The subtracted background was 2,500 cpm.

lum are regarded functionally equivalent with respect to their translocation capacity.

We have previously shown that two transmembrane glycoproteins, ribophorins I and II are found in RM but are virtually absent from SM prepared from several organs of different species (20, 21, 28). In the following paper (27) we demonstrate that the ribophorin content of microsomal subfractions correlates closely with their ribosome content and that the molar ratio of both components is about one. These results support a model for the ER in which the translocation apparatus is segregated only to the rough domains of this continuous endo membrane system.

Although the capacity of stripped microsomal subfractions to rebind ribosomes in vitro correlated well with their ribosome content stoichiometric correlations could not be established for their ability to effect in vitro the processing and co-translational segregation. In this case removal of native ribosomes from microsomal subfractions did not alter significantly their translocation activity, which suggests that only sites that in the native membranes are not occupied by ribosomes anchored by their nascent chains are active in in vitro translocation. The clear correlation between the translocation capacity of microsomal subfractions and RNA content may therefore reflect the presence in each subfraction of a small, but fixed percentage of unoccupied, and therefore potentially active translocation sites. That the generally applied stripping procedures are not effective in activating translocation sites occupied by a membrane bound ribosomes is not entirely surprising since at moderately high salt concentrations the chelating agents used fail to remove a significant fraction of large ribosomal subunits and peptidyl-tRNA (36). That indeed only sites that become available after natural termination are translocation competent was suggested (19)

by experiments in which following a block of initiation in vivo a higher percentage of translocation competent vesicles was found in a smooth microsomal fraction. On the other hand, the translocation and processing activity found in rat liver SM was markedly reduced when cycloheximide was administered to animals before sacrifice (19). Therefore the previous physiological state of the cell may affect significantly the co-translational translocation and processing activity in vitro of isolated microsomal membranes.

We thank Dr. D. D. Sabatini and Dr. H. Beaufay for their support and help throughout the work. The help of Mr. Gerald Schnittman and Mr. Douglas Russell for technical expertise, of Ms. Heide Plesken, Mr. B. Zeitlow and Ms. J. Culkin for preparing illustrations and the photographic work, and of Mrs. M. Cort and T. Singfield for typing the manuscript is greatly appreciated.

This work was supported by a grant from the Services de Programmation de la Politique Scientifique, Belgium and GM 21971 from the National Institutes of Health and by grants from the Belgian Funds de la Recherche Fondamentale Collective. G. Kreibich is the recipient of an Irma T. Hirschl Research Career Award.

Received for publication 14 June 1984, and in revised form 13 August 1984.

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