Subcellular Distribution of Signal Recognition Particle and 7SL-RNA Determined with Polypeptide-Specific Antibodies and Complementary DNA Probe

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ABSTRACT Signal recognition particle (SRP) is a ribonucleoprotein consisting of six distinct polypeptides and one molecule of small cytoplasmic 7SL-RNA. The particle was previously shown to function in protein translocation across and protein integration into the endoplasmic reticulum membrane.

Polypeptide specific antibodies were raised in rabbits against the 72,000-, 68,000-, and 54,000-mol-wt polypeptide of SRP. All three antibodies are shown to neutralize SRP activity in vitro. A solid phase radioimmune assay is described and used to follow SRP in various cell fractions. The partitioning of SRP is shown to be dependent on the ionic conditions of the fractionation. Under conditions approximating physiological ionic strength, SRP is found to be about equally distributed between a membrane associated (38%) and a free (15%) or ribosome associated (47%) state. Furthermore, it is shown that >75% of the total cellular 7SL-RNA is associated with SRP polypeptide in these fractions. Thus it is likely that the major—if not the only—cellular function of 7SL-RNA is as a part of SRP.

Signal recognition particle was shown to function as the cellular adapter between the cytoplasmic protein translation apparatus and the membrane-bound protein translocation machinery of the endoplasmic reticulum. In the cytoplasm signal recognition particle (SRP)[1] interacts with ribosomes (1). After these ribosomes get engaged in the synthesis of secretory (1, 2), lysosomal (3), or certain classes of integral membrane proteins (4), SRP arrests the elongation of the nascent polypeptide chains just after their signal sequences become exposed on the surface of the ribosome (5). This elongation arrest is reversed once the ribosome interacts through the tightly bound SRP with a specific integral membrane protein of the endoplasmic reticulum, the SRP-receptor (6, 7) (or docking protein [8, 9]). As a result, the ribosome forms a functional ribosome-membrane junction (10), allowing the vectorial translocation of the nascent polypeptide chain across the lipid bilayer (11).

[1] *Abbreviations used in this paper:* cDNA, complementary DNA; DTT, dithiothreitol; IgG, immunoglobulin G; PBS, phosphate-buffered saline; PL, prolactin; pPL, preprolactin; SRP, signal recognition particle; SSC, 15 mM NaCitrate, 150 mM NaCl, pH 7.0; TEA, triethanolamine · HOAc, pH 7.5.

SRP consists of six distinct polypeptide chains (12) and one molecule of small cytoplasmic 7SL-RNA (13). Here we describe the preparation of specific antibody against three of the polypeptide components of SRP and their use to study its function and subcellular distribution.

MATERIALS AND METHODS

Preparation of Antibodies against Purified SRP Polypeptides: SRP was denatured by boiling in SDS and SRP polypeptides were fractionated by chromatography on hydroxylapatite (HTP; Biorad Laboratories, Richmond, CA) (14). The column was developed with a linear gradient of 10 column volumes of 50–400 mM Na phosphate (pH 6.5), 0.1% SDS, 1 mM dithiothreitol. The elution position of SRP polypeptides was determined by subjecting aliquots of the column fractions to PAGE in SDS. The 68,000-mol-wt SRP polypeptide could be well separated from the 72,000- and 54,000-mol-wt polypeptides. The peak fractions were trichloroacetic acid–precipitated, subjected to preparative PAGE in SDS and eluted from the gel as described (15). The eluates were concentrated on a small hydroxylapatite column and checked for purity on PAGE (see Fig. 1, lanes *b–e*). Antibodies were raised in white New Zealand rabbits against the 72,000-mol-wt, the 68,000-, and the 54,000-mol-wt SRP polypeptide. About 50 μg of each polypeptide were injected as emulsion in complete Freund's adjuvant directly into lymph nodes. Rabbits were boosted twice at 10-d intervals with 50–100 μg antigen each, after which time a stable titer was reached (as assayed by dot blots [16]).

Polypeptide specific IgGs were prepared by immunoselection of the sera on

Sepharose columns to which the corresponding purified SRP polypeptide were covalently attached. To a 2-ml aliquot of serum a solution of 4 M NaCl and a solution of 10% Triton X-100, 2% SDS was added to yield final concentrations of 500 mM NaCl (taking into consideration that the serum contained ~150 mM NaCl), 0.1% Triton X-100, 0.02% SDS. The serum was then recycled for 10 times over a 0.5-ml column of Sepharose Cl-4B having ~50 μg of purified SRP polypeptide bound to it. The column was washed with 2.5 ml of solution A (20 mM Na phosphate, pH 7.5, 500 mM NaCl, 0.1% Triton X-100, 0.02% SDS) followed by 2.5 ml of solution A, but without detergents. Bound IgG was eluted with 0.5 ml of a solution of 50 mM glycine. HCl (pH 2.3), 500 mM NaCl, followed by 0.5 ml of solution A without detergents, followed by 0.5 ml of a solution 3.0 M NH_4SCN, 50 mM Na phosphate, pH 7.5, followed by 1 ml of solution A without detergents. The eluates were collected on ice into a siliconized Corex tube containing 100 μl of a solution of 0.5 M Na_2HPO_4 to neutralize the first elution buffer. IgG was precipitated by the addition of an equal volume of saturated $(NH_4)_2SO_4$ (30 min on ice) and collected by centrifugation for 30 min at 10,000 *g*. The pellet was dissolved in a small volume of PBS; the solution was precipitated with $(NH_4)_2SO_4$ and the precipitate was finally dissolved in PBS containing 50% glycerol, 0.02% NaN_3 and stored at −20°C. The protein concentration was determined by the method of Schaffner and Weissmann (17). When IgG fractions were to be added to in vitro translation systems, the phosphate buffer was first exchanged by gel filtration to a buffer containing 50 mM triethanolamine · HOAc, pH 7.5, (TEA), 150 mM KOAc, 10% glycerol.

Transfer of Proteins to Nitrocellulose and Detection with Antibody: Gel blots were set up and the antigens were detected as described by Fisher et al. (16) with the following modification. Immunoselected IgG fractions were used throughout this study. We usually employed 50 ng immunoselected IgG per lane of the SDS polyacrylamide gel to be analyzed. Detection of bound antibody was performed with iodinated second antibody (goat-anti-rabbit IgG, [Cappel Laboratories, Cochranville, PA] labeled with ^{125}I Bolton-Hunter reagent (18) to ~4 μCi/μg specific activity). After incubation with second antibody the nitrocellulose sheets were washed and dried as described and then autoradiographed using an intensifying screen. Radioactive bands were localized by aligning the film and the gel, they were excised from the gel and their radioactivity was determined by gamma counting.

Transfer of RNA to Nitrocellulose and Detection with Cloned cDNA: Samples up to 10 μg total nucleic acid were electrophoresed on 2 mm thick 1% agarose gels containing 2.2 M HCHO in 20 mM 3-[N-morpholino]propanesulfonic acid, 5 mM NaOAc, 0.1 mM EDTA at pH 7.0 (19). After electrophoresis the gels were soaked in 20 times 15 mM Na citrate, 150 mM NaCl, pH 7.0 (SSC) for 30 min at room temperature, blotted onto nitrocellulose over night using 10 times SSC as transfer buffer. The nitrocellulose filters were washed in 2 times SSC and baked at 80°C for 2 h at reduced pressure.

The nitrocellulose sheet was prehybridized for 2 h in a solution of 50% formamide, 5 times SSC, 5 times Denhardt's solution (contributes 100 μg/ml Ficoll, 100 μg/ml polyvinylpyrrolidone, 100 μg/ml bovine serum albumin [fraction V] final concentration), 100 μg/ml transfer RNA, 100 μg/ml sonicated herring sperm DNA. Hybridization was at 37°C for 15 h using ~10^6 cpm of nick-replicated plasmid per gel lane. The filter was washed 3 times for 30 min in a large volume of 2 times SSC containing 0.1% SDS, dried, and autoradiographed using an intensifying screen. Radioactive bands were localized by superimposing film and nitrocellulose sheet, excised, and their radioactivity determined by scintillation counting.

RESULTS

Antibodies against SRP Polypeptides

To raise antibodies specific to individual SRP polypeptides, we first denatured purified SRP in SDS and then applied separation techniques in SDS to fractionate the polypeptides. We found it sufficient to chromatograph the polypeptide mixture on hydroxylapatite columns (14) and then to subject the peak fractions to preparative PAGE in SDS. As shown in Fig. 1, lanes *b–d*, we were able to obtain fractions of the larger SRP polypeptides that showed no detectable cross-contamination. These fractions were used as antigen in immunization and in immunoselection procedures (see Materials and Methods) to obtain high titer, monospecific IgG fractions. When a total pancreatic protein profile was blotted onto nitrocellulose (Fig. 1, lane *f*) and probed with these IgG fractions, only one band of the same molecular weight as the corresponding SRP polypeptide was detected (Fig. 1, lanes *h–j*). It is apparent that these three polypeptides were not cross-reactive, nor did the antibodies detect any of the three small molecular weight SRP polypeptides. Antigens of the same molecular weight could also be detected in different tissues of various mammalian species, namely dog liver (Fig. 2, lanes *a–c*), bovine pituitary (Fig. 2, lanes *d–f*), human placenta (Fig. 2, lanes *g–i*) and rabbit reticulocytes (E. Evans and P. Walter, unpublished observations). In bovine pituitary as well as human placenta the anti-54,000-mol-wt antibody also detected some minor bands, in addition to the 54,000-mol-wt polypeptide (Fig. 2, lanes *f* and *i*). At present, we cannot distinguish whether these bands arise from breakdown of 54,000-mol-wt SRP polypeptide and/or additional cross-reactive proteins in these tissues. No SRP cross-reactive polypeptides were detected in amphibian, insect, plant, yeast, or procaryotic total cell lysates (data not shown).

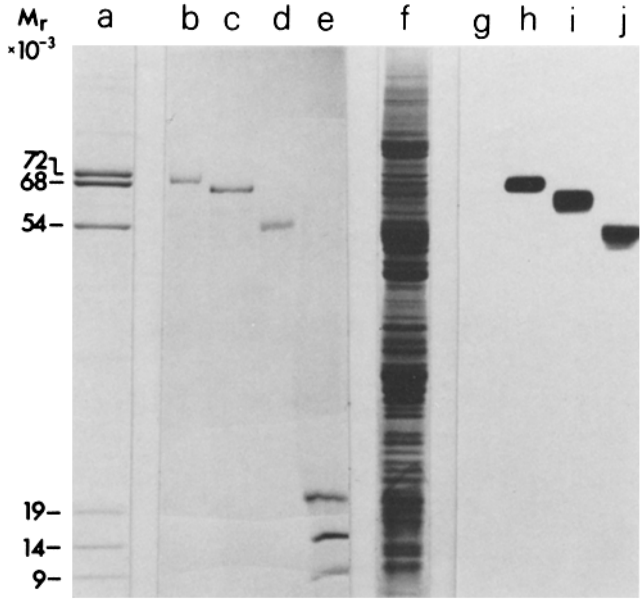

FIGURE 1 Characterization of rabbit antibodies to SRP polypeptides. SRP (lane *a*) was denatured in SDS and fractionated into individual polypeptides as described in Materials and Methods. Aliquots of purified SRP polypeptide fractions are shown in lanes *b–e*. No attempts have yet been made to fractionate the 19,000-, 14,000-, and 9,000-mol-wt polypeptides (lane *e*) or to raise antibodies against them. Immunoselected IgG raised against the 72,000- (lane *b*), the 68,000- (lane *c*), and the 72,000-mol-wt polypeptide (lane *d*) were prepared as described in Materials and Methods and used as probes on nitrocellulose blots (lanes *h*, *i*, and *j*). Total pancreatic protein (200 μg per lane) was fractionated by PAGE in SDS (lane *f*) and blotted onto nitrocellulose. Strips were probed with nonimmune IgG (lane *g*), anti-72,000-mol-wt IgG (lane *h*), anti-68,000-mol-wt IgG (lane *i*), or anti-54,000-mol-wt IgG (lane *j*), respectively. 50 ng of immunoselected IgG was used per strip, bound IgG was detected by incubating with ^{125}I goat-anti-rabbit IgG (50,000 cpm/strip) followed by autoradiography. Lanes *a–f* are Coomassie-Blue-stained 10–15% gradient polyacrylamide gels. Lane *g–j* are an autoradiogram of the nitrocellulose blots (8-h exposure). The molecular weights (M_r) of SRP polypeptides are indicated.

Antibodies against SRP Polypeptides Neutralize SRP Activity In Vitro

When antibodies against the individual SRP polypeptides were titrated into an in vitro protein translocation assay (20)

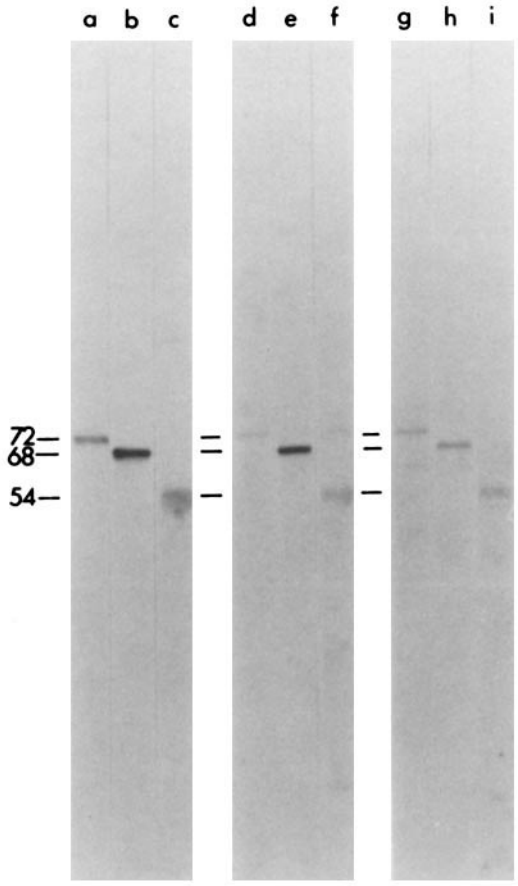

FIGURE 2 Detection of SRP polypeptides in different tissues and species. Canine liver (lanes *a–c*), bovine pituitary (lanes *d–f*), and human placenta (lanes *g–i*) were frozen in liquid N_2 and ground to a fine powder. The frozen material was then added to boiling solution of 1% SDS, 100 mM Na phosphate, 50 mM dithiothreitol and homogenized in a Dounce homogenizer after sonication to shear DNA to reduce the viscosity. The material was precipitated with trichloroacetic acid and prepared for PAGE in SDS. About 200 μg of protein were loaded per lane. After PAGE the proteins were transferred to nitrocellulose. Strips were probed with immunoselected anti-72,000-mol-wt IgG (lanes *a*, *d*, and *g*), anti-68,000-mol-wt IgG (lanes *b*, *e*, and *h*), or anti-54,000-mol-wt IgG (lanes *c*, *f*, and *i*), respectively. Bound IgG was detected by autoradiography after incubation of the strips with ^{125}I goat-anti-rabbit IgG. 50 ng of immunoselected first antibody and 50,000 cpm of second antibody were used per strip. The molecular weight (10^{-3}) for the relevant SRP polypeptides is indicated.

the following data were obtained (see Fig. 3). In each of the three cases an inhibition of the protein translocation process was observed as the IgG concentration was raised. These results indicate that all three IgG fractions were able to recognize native SRP (even though they were raised against SDS denatured polypeptides) and to block the function of the particle. As a result, the SRP-dependent process of protein translocation could no longer take place. All three of the specific IgG fractions caused <30% inhibition on protein synthesis in the absence of RM (data not shown).

Apparently only a fraction of the IgG molecules (raised and immunoselected against SDS-denatured polypeptides) was capable of binding to native SRP. This explains the excess of IgG over SRP required to block translocation (at 100 μg/ml IgG is in about 20-fold molar excess over SRP in these reactions). In addition, some of the IgG might have been irreversibly denatured in the immunoselection step (see below).

It is interesting to note that while increasing concentrations of IgG, in all three cases, led to a decrease in the synthesis of translocated prolactin (PL) only the presence of anti-54,000-mol-wt IgG did not cause a concomitant increase in preprolactin (pPL) synthesis. This result might imply that while anti-72,000-mol-wt IgG and anti-68,000-mol-wt IgG blocked some of the early functions of SRP, the anti-54,000-mol-wt IgG apparently still allowed SRP-dependent elongation arrest of pPL synthesis to take place, but interfered with the subsequent release of this arrest by SRP receptor. We have attempted to reconfirm these results using Fab fragments of IgG fractions, to avoid possible artifacts due to cross-linking of individual SRP molecules with divalent IgG. However, so far we have failed to produce Fab fragments with detectable activity. In our hands, the immunoselected IgG fractions tended to precipitate irreversibly in the papain digestion step. The effect might have been caused by the considerable dilution and/or partial denaturation in the immunoselection step. This step was, however, required to produce IgG of sufficient specific activity to show an effect in the in vitro assay. Thus, although we could demonstrate that all three IgG fractions inhibit SRP activity, the above mentioned molecular details of this inhibition have to be interpreted with caution, due to the lack of monovalent Fab fragments as probes.

Distribution of SRP during Cell Fractionation

The insert in Fig. 4 shows that the blot assay as used in Figs. 1 and 2 can be used in a quantitative way, such that the signal of second antibody bound (measured in counts per minute in a specific band on the nitrocellulose blot) is directly proportional to the amount of antigen present in the original sample. We have used this assay to follow the 68,000-mol-wt SRP polypeptide as a representative for SRP through the cell

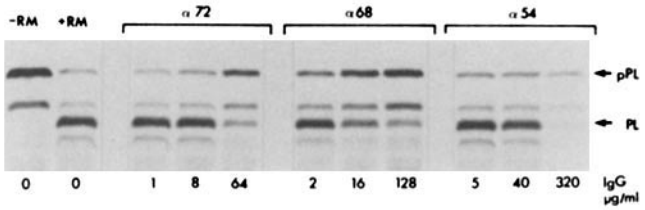

FIGURE 3 Antibodies to SRP-polypeptides block protein translocation in vitro. Bovine pituitary RNA was translated in a cell-free wheat germ translation system (lane *–RM*). Translation products were separated by PAGE in SDS and visualized by autoradiography. The band obtained correspond to preprolactin (*pPL*) and pregrowth hormone (faint band below *pPL*). If the translation system was supplemented with two equivalent units (12) of dog pancreatic rough microsomes (*RM*), conversion of *pPL* to authentic prolactin (*PL*) and conversion of pregrowth hormone to growth hormone (faint band below *PL*) was observed (lane *+RM*) due to the translocation of the nascent chain across the microsomal membrane and concomitant removal of the signal peptide by signal peptidase (12). The remaining lanes show the effect of increasing concentrations (indicated at the bottom) of immunoselected anti-SRP IgG (anti-72,000-, anti-68,000-, and anti-54,000-mol wt IgG) in this assay. The IgG fractions were mixed first with rough microsomes on ice and then the other components were added. Nonimmune IgG had no effect on translation at 50 μg/ml. To avoid reduction of the IgG molecules, dithiothreitol was removed from the wheat germ system and the rough microsome fraction by gel filtration and replaced by glutathione (final concentration 5 mM).

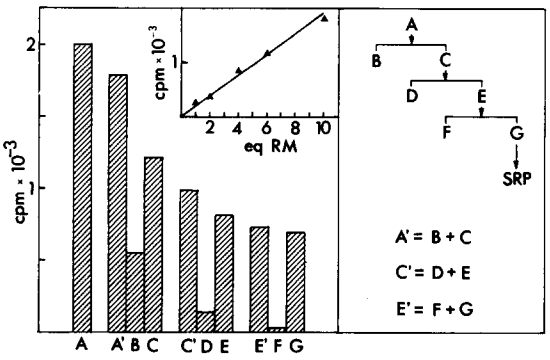

FIGURE 4 Distribution of SRP during cell fractionation. Detection of the 68,000-mol-wt SRP-polypeptide using immunoselected anti-68,000-mol-wt IgG on nitrocellulose blots (see Materials and Methods) was used to quantitate the distribution of SRP during cell fractionation of dog pancreas. Cell fractionation was carried out as described (20), equivalent aliquots of the various fractions were precipitated by trichloroacetic acid, subjected to PAGE in SDS and transferred to nitrocellulose. The anti-68,000-mol-wt SRP polypeptide was detected by incubating the blot with 68,000-mol-wt IgG (50 μg/lane), followed by ^{125}I rabbit anti goat IgG (50,000 cpm/lane) and subsequent autoradiography. The autoradiograph was used to localize the bands on the nitrocellulose sheet. The bands were excised and their radioactivity determined by gamma counting. The *inset* shows a titration of rough microsomes (*RM*) to demonstrate that the assay is linear. The fractions analyzed (see reference 20 for detailed conditions for cell fractionation) were homogenate (*A*), nuclear pellet (*B*), postnuclear supernatant (*C*) (after 10 min at 1,000 *g*), mitochondrial pellet (*D*), postmitochondrial supernatant (*E*) (after 10 min at 10,000 *g*), high-speed supernatant (*F*) and rough microsomes (*G*) (after 2.5 h at 140,000 *g*). The fractionation is diagrammatically displayed in the right hand panel. To show the recovery of SRP, the sums of the fractions resulting in each fractionation step are given (*A'*, *C'*, and *E'*) and should be compared to the corresponding starting material (*A'* to *A*, *C'* to *C* and *E'* to *E*, respectively).

fractionation scheme that is generally used by us to prepare dog pancreatic microsomal membranes (Fig. 4). We estimated from the data shown in Fig. 4 that we recovered ~35% of the SRP present in the homogenate (Fig. 4*A*) in the rough microsomal fraction (Fig. 4*G*), whereas 27% were lost in the nuclear pellet (Fig. 4*B*), 7% in the mitochondrial pellet (Fig. 4*D*) and only 2% were left in the final cytoplasmic supernatant (Fig. 4*F*). Another 30% were unaccounted for, representing the additive losses in the three subsequent fractionation steps (Fig. 4, compare *A'* to *A*, *C'* to *C*, and *E'* to *E*).

To address questions of the in vivo distribution of SRP between the different cellular structures with which SRP is interacting functionally, we decided to investigate its associations in the postmitochondrial supernatant (Fig. 4*E*) more closely. For this purpose, we fractionated the postmitochondrial supernatant further on sucrose gradients (Fig. 5). Since it was known that the interaction of SRP with rough microsomal membranes is salt-dependent (12, 21), we performed this gradient analysis at different salt concentrations. When the UV profile of the sucrose gradients was analyzed (Fig. 5,*A–E*), three distinct peaks could be distinguished, one on the top of the gradient corresponding to a cytoplasmic fraction, a second corresponding to 80S monomeric ribosomes possibly containing a tail of partially degraded free polysomes and a third peak corresponding to the rough microsomal membrane fraction. SRP was quantitated measuring the amount of 68,000-mol-wt SRP polypeptide in fractions across the gradients (Fig. 5,*F–J*). From these data (summarized in Fig. 5*K*) it was apparent that at low salt concentrations (50 mM KOAc) most of SRP was membrane-associated. At salt concentrations approximating physiological conditions (150 mM KOAc) some SRP dissociated from the membrane such that an about equal distribution between membrane-bound SRP (38%) and ribosome-associated SRP (47%), as well as a

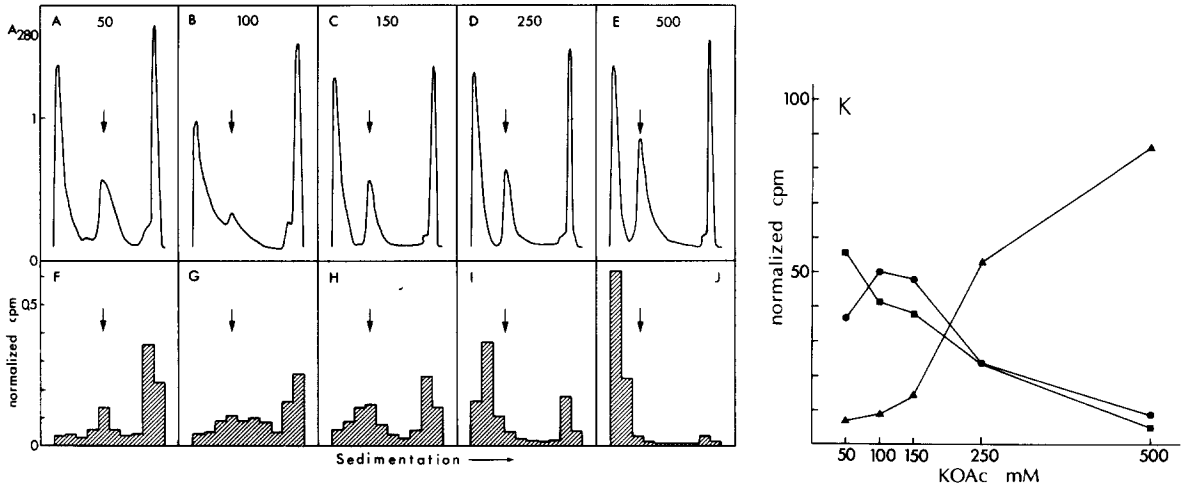

FIGURE 5 Distribution of SRP as a function of ionic strength. Aliquots of postmitochondrial supernatant (fraction E in Fig. 4) were adjusted to various KOAc concentrations (see below) and layered on top of a 10–30% sucrose gradient in 50 mM TEA, 6 mM $Mg(OAc)_2$, 1 mM EDTA, 1 mM dithiothreitol, containing KOAc at the following concentrations: 50 mM (*A* and *F*), 100 mM (*B* and *G*), 150 mM (*C* and *H*), 250 mM (*D* and *I*), and 500 mM (*E* and *J*). The gradient tubes also contained a 1.0-ml cushion of 70% sucrose in the same buffers at the bottom to prevent microsomes from pelleting. Gradients were centrifuged for 100 min at 4°C at 40,000 rpm in a Beckman SW40 rotor. They were fractionated using an ISCO gradient fractionator and the absorbance at 280 nm was recorded (*A–E*). Ten equally sized fractions were collected. Polypeptides were trichloroacetic acid-precipitated, subjected to PAGE in SDS and the amount of 68,000-mol-wt SRP polypeptide, was determined as described in Fig. 4. The cpm values were normalized, such that the sum of all fractions of each gradient equaled 1. The position of the 80S monomeric ribosome is indicated with arrows. In *K* the distribution of SRP is plotted as a function of the salt concentration. Three cellular fractions were defined as follows: free SRP (▲) as the sum of the SRP contained in the two top fractions of the gradients; membrane-bound SRP (■) as the SRP contained in the two bottom fractions; and ribosome-bound SRP (●) as the SRP contained in the six middle fractions. Cpm value are given as percentage of SRP in one of these three fractions.

significant level of free SRP (15%) was observed. When the salt concentration was raised further, SRP was found to readily dissociate from both ribosomes and rough microsomes, and could be almost quantitatively recovered in the cytoplasmic fraction.

These data confirm the rationale behind the cell fractionation scheme that generally precedes the purification of SRP. A rough microsomal membrane fraction is prepared in under low salt (50 mM KOAc) conditions and SRP is subsequently extracted from these microsomes in a 500 mM salt-wash.

Correlation of 68,000-mol-wt SRP Polypeptide with 7SL-RNA

Using the data described in Figs. 4 and 5 we designed a fractionation scheme for canine pancreas that would render cellular SRP soluble in the initial homogenate and would yield a quantitative recovery (Fig. 6*F*). The rationale was to salt-extract SRP already at the stage of the homogenate from all cellular structures and then to remove these organelles by differential centrifugation. Nuclei were removed first in a low speed spin to avoid breakage and the release of DNA. The nuclear fraction was reextracted with 500 mM KOAc and the extract was combined with the postnuclear supernatant. All remaining material with a sedimentation coefficient larger than 50S was removed from this supernatant during a high speed centrifugation step.

The analysis of the resulting three fractions, the homogenate H, the high-salt pellet fraction P, and the high-salt supernatant S is shown in Fig. 6. The polypeptide profile (Fig. 6*A*) was dominated by the massive amounts of secretory proteins present in the secretory vesicles of the tissue. These major bands were also dominant in fraction S, presumably due to the breakage of some of the secretory granules. That a good fractionation was obtained nevertheless was apparent from the distribution of nucleic acid (Fig. 6*B*). Whereas all of the DNA and ribosomal RNA was shown to pellet (present in fractions H and P), the majority of 7S RNA and transfer RNA was recovered in fraction S.

To correlate the cell fractionation behavior of SRP-poly-

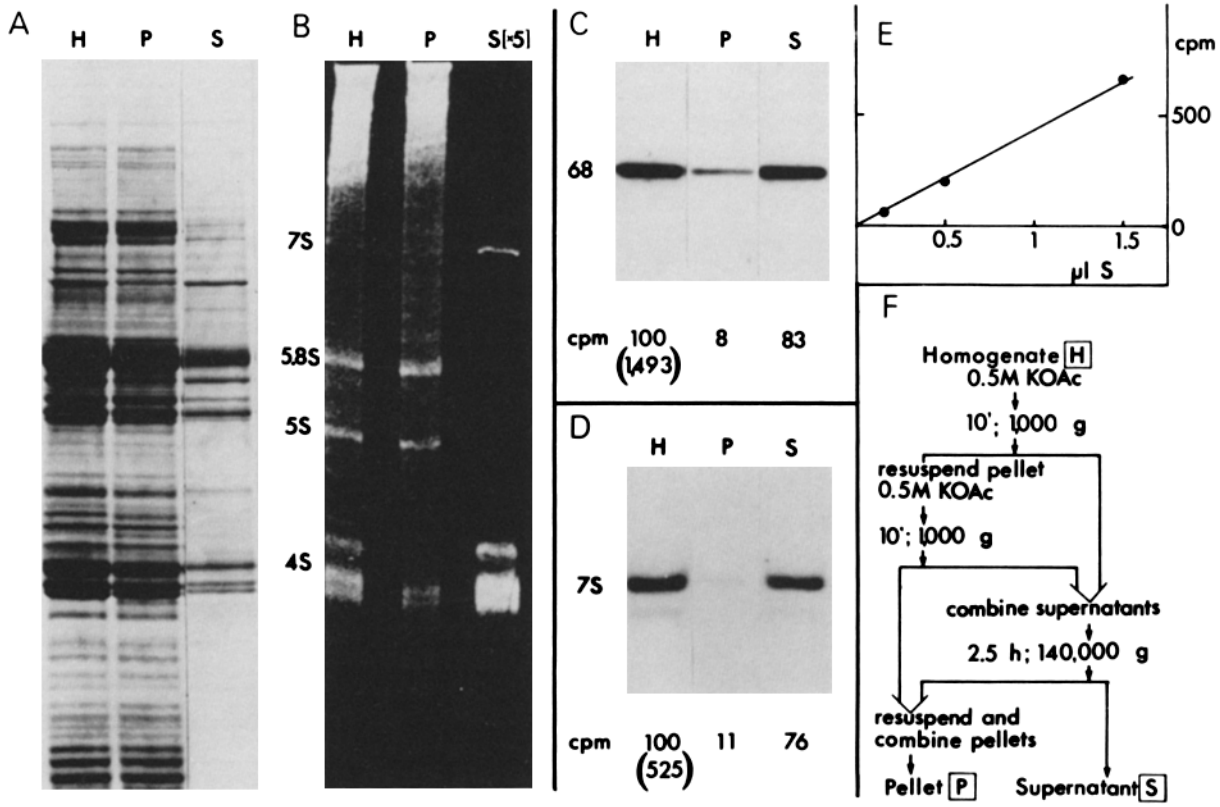

FIGURE 6 Cell fractionation in high-salt buffer. Dog pancreas was homogenized as previously described (20). A solution of 4 M KOAc was added to the homogenate on ice to yield a final concentration of 0.5 M KOAc. This "high-salt homogenate" (*H*) was fractionated as outlined in *F* to yield two fractions, a high-salt pellet fraction (*P*) and a high-salt supernatant fraction (*S*). Equivalent aliquots of each fraction (*H*, *P*, and *S*) were TCA-precipitated and analyzed by PAGE in SDS as described in Fig. 1. (*A*) A Coomassie-Blue-stained gel is shown. Lane *H* is directly comparable to lane *f* in Fig. 1. Separate aliquots were ethanol-precipitated and nucleic acid was prepared from the pellet by proteinase K treatment and perchlorate precipitation (29). Equivalent aliquots of the nucleic acid prepared from *H* and *P* and five times the amount of *S* were analyzed by PAGE in 7 M urea and visualized by staining in ethidium bromide (*B*). The positions of tRNA (4S), ribosomal 5S and 5.8S RNA and 7SL-RNA (7S) are indicated. The amount of 68,000-mol-wt SRP polypeptide in fractions *H*, *P*, and *S* was quantitated as described in Fig. 4. The autoradiogram of the nitrocellulose blot is shown in C. Cpm values were normalized to be 100 for fraction H (1,493 cpm) and are indicated at the bottom of each lane. The amount of 7SL-RNA in fractions *H*, *P*, and *S* was determined as described in Materials and Methods. *D* shows the autoradiogram of the nitrocellulose blot using nick-translated S-fragment of a cDNA clone of 7SL-RNA (see Results and [22]) as the probe. Bands were excised from the nitrocellulose and their radioactivity determined by scintillation counting. The cpm values were normalized to be 100 for fraction H (525 cpm) and are given below each lane. *E* shows a titration of fraction S in this assay to demonstrate the linearity of the response.

peptides with that of 7SL-RNA, we used two specific probes to quantitate these components. The 68,000-mol-wt SRP polypeptide was almost quantitatively recovered (90%) fraction S, as estimated by probing with anti-68,000-mol-wt IgG as described above (Fig. 6*C*). 7SL-RNA was quantitated on a nitrocellulose blot of a formaldehyde-agarose gel that was probed by hybridization (19) with a cloned restriction fragment of 7SL-RNA sequences. This clone (residues 105 to 230 of 7SL-RNA [22]) was kindly provided to us by Elizabetta Ullu and was devoid of the regions in 7SL-RNA which are homologous to the repetitive ALU-sequence family. It is thus a unique and specific probe for 7SL-RNA and does not cross-hybridize with other ALU-like transcripts. Fig. 6*E* shows that this solid phase hybridization assay resulted in a linear response curve. We quantitated the amount of 7SL-RNA in the three cell fractions and, as shown in Fig. 6*D*, we were able to demonstrate that ~87% of all detectable cellular 7SL-RNA partitioned into fraction S.

To directly demonstrate that all 7SL-RNA contained in fraction S is indeed associated with SRP polypeptides (and thus most likely contained in SRP) we performed the immunoprecipitation experiments shown in Fig. 7. It is apparent that under the native immunoprecipitation conditions used, anti-68,000-mol-wt IgG was capable to bind and thus to precipitate 87% of 7SL-RNA (Fig. 7*C*) that were contained in fraction S (Fig. 6). This immunoprecipitation was shown to be specific, because nonimmune IgG did not precipitate

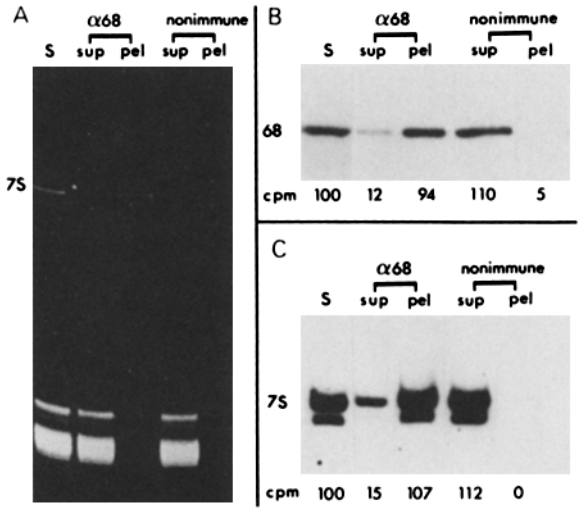

FIGURE 7 Immunoprecipitation of 7SL-RNA. The high-salt supernatant fraction S (see Fig. 6) was diluted with 2 vol of water to reduce the salt-concentration. Immunoselected anti-68,000-mol-wt IgG or an identical amount of nonimmune IgG was bound to protein A Sepharose. The diluted S was incubated for 1 h at 4°C with the IgG coated Sepharose beads on an overhead shaker. The beads were then pelleted, yielding an immunoprecipitated fraction (*pel*) and a supernatant fraction (*sup*). Nucleic acid was extracted from these fractions and analyzed (see Fig. 6) by PAGE in 7 M urea (*A*). The relative amounts of 7SL-RNA were quantitated (*C*) as described in Materials and Methods and in Fig. 6. Authentic 7SL-RNA comigated with the upper of the two bands that are visible on the autoradiogram. The lower band was most likely a breakdown product. It amounted to <25% of the cpm in both bands and was excluded from the quantitative interpretation indicated below the lanes. The relative amount of 68,000-mol-wt SRP polypeptide present in these fractions was quantitated (*B*) as described in Figs. 4 and 6.

68,000-mol-wt SRP polypeptide (Fig. 7*B*) or 7SL-RNA (Fig. 7*C*). Furthermore, all stained transfer RNA (Fig. 7*A*), as well as all stained protein (which was not obscured by the IgG polypeptide chains) was not immunoprecipitated with anti-68,000-mol-wt IgG (data not shown).

Interestingly, *all* stainable 7S RNA in fraction S was immunoprecipitable with anti-68,000-mol-wt IgG (Fig. 7*A*), indicating that all of it corresponds to 7SL-RNA. This finding allowed us to generalize the fractionation scheme shown in Fig. 6*F* and to prepare essentially pure 7SL-RNA from other sources, e.g., *Xenopus laevis* liver and *Drosophila melanogaster* embryos (23). In each case we obtained a major band in the corresponding fraction S which co-migrated with canine 7SL-RNA on polyacrylamide gels in 7 M urea. When eluted after preparative PAGE all of the RNA contained in these bands could be reconstituted with mammalian SRP polypeptides to form active SRP (23). Thus, all 7S RNA in fraction S obtained from a variety of sources appeared to be functionally equivalent to 7SL-RNA.

DISCUSSION

Using polypeptide-specific antibodies against three of the six polypeptides of SRP, we have demonstrated here the existence of cross-reactive proteins of identical molecular weight in a variety of tissues and species. Although these analyses were limited to mammalian species due to the particular cross-reactivity properties of the antibodies, many lines of evidence indicate that SRP or analogous particles exist in all eucaryotic and maybe even procaryotic cells. Most intriguing are experiments that demonstrate the wide interspecies interchangeability of the various functional components of the translation/translocation machineries in in vitro assays. The most heterogeneous "in vitro chimeras" that have been shown to work with fidelity include (*a*) procaryotic secretory proteins synthesized on plant ribosomes, recognized by mammalian SRP and translocated across mammalian membranes (24) and (*b*) the demonstration that functional SRP themselves could be reconstituted from mammalian SRP protein and either amphibian or insect 7SL-RNA (23).

The intracellular distribution of SRP as determined by cell fractionation (Fig. 5) reflects its potential to interact with various cellular components. In particular, SRP was proposed to cycle between a cytoplasmic and a membrane-bound state (5). In the cytoplasm at least three different states can be distinguished (1): (*a*) a free form, (*b*) a form loosely bound to biosynthetically inactive ribosomes, and (*c*) a tight interaction with ribosomes involved in the synthesis of secretory proteins. At physiological ionic strength an about even distribution of SRP between the cytoplasmic and the membrane-bound state was observed. A significant amount of the cytoplasmic SRP appeared to be free. Depending on the ionic conditions, a different partitioning of SRP into subcellular fractions can be achieved. These results may explain some of the confusing data on the subcellular distribution of 7SL-RNA that have previously accumulated in the literature (25–28).

Using the SRP specific antibodies in a combination of radioimmunoassay and immunoprecipitation, we demonstrated here that >75% of the total cellular 7SL-RNA is associated with the 68,000-mol-wt SRP polypeptide that functions as the antigen. We can account only for 75% of the 68,000-mol-wt SRP polypeptide and thus the likely possibility remains that most of the unaccounted 7SL-RNA is also associated with SRP polypeptides. We therefore conclude,

that the main—if not the only—function of 7SL-RNA is as part of SRP. We can, of course, not exclude the possibility that SRP itself functions in more aspects of translation or protein translocation than have been described so far.

We thank Dr. Wolf-Dieter Schleuning for his help in the preparation of the antibodies, Dr. Paul Fisher for his advice concerning the blotting techniques, and Dr. Elizabetta Ullu for making the clones of 7SL-RNA available to us, as well as her advice in the hybridization techniques.

This work was supported by National Institutes of Health grant GM-27155.

Received for publication 28 June 1983, and in revised form 19 August 1983.

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