

# The Signal Recognition Particle Receptor Is a Complex That Contains Two Distinct Polypeptide Chains

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**Abstract.** Signal recognition particle (SRP) and SRP receptor are known to be essential components of the cellular machinery that targets nascent secretory proteins to the endoplasmic reticulum (ER) membrane. Here we report that the SRP receptor contains, in addition to the previously identified and sequenced 69-kD polypeptide ( $\alpha$ -subunit, SR $\alpha$ ), a 30-kD  $\beta$ -subunit (SR $\beta$ ).

When SRP receptor was purified by SRP-Sepharose affinity chromatography, we observed the co-purification of two other ER membrane proteins. Both proteins are  $\sim$ 30 kD in size and are immunologically distinct from each other, as well as from SR $\alpha$  and SRP proteins. One of the 30-kD proteins (SR $\beta$ ) forms a tight complex with SR $\alpha$  in detergent solution that is

stable to high salt and can be immunoprecipitated with antibodies to either SR $\alpha$  or SR $\beta$ . Both subunits are present in the ER membrane in equimolar amounts and co-fractionate in constant stoichiometry when rough and smooth liver microsomes are separated on sucrose gradients. We therefore conclude that SR $\beta$  is an integral component of SRP receptor. The presence of SR $\beta$  was previously masked by proteolytic breakdown products of SR $\alpha$  observed by others and by the presence of another 30-kD ER membrane protein (mp30) which co-purifies with SR $\alpha$ . Mp30 binds to SRP-Sepharose directly and is present in the ER membrane in several-fold molar excess of SR $\alpha$  and SR $\beta$ . The affinity of mp30 for SRP suggests that it may serve a yet unknown function in protein translocation.

**N**ASCENT secretory proteins are targeted specifically to the endoplasmic reticulum (ER)<sup>1</sup> membrane. Two components, the signal recognition particle (SRP) and the SRP receptor (or docking protein [23]), are known constituents of the cellular targeting apparatus responsible for this protein sorting event (30). SRP binds to signal sequences within the nascent polypeptide chain as it emerges from the ribosome (18, 19) and causes an arrest or pause of protein synthesis. Then, when the ribosome-bound SRP interacts with the ER membrane, the elongation arrest is released (27). The ribosome engages in a functional ribosome-membrane junction that translocates the growing polypeptide chain across the membrane by a mechanism that is, as yet, poorly understood.

The SRP receptor was functionally defined as an activity residing in a microsomal membrane protein that would release the elongation arrest (27). This activity was purified by SRP-Sepharose affinity chromatography and attributed to a 69-kD ER membrane protein (12), which we will henceforth refer to as the  $\alpha$ -subunit of the SRP receptor (SR $\alpha$ ). Independent evidence for the involvement of SR $\alpha$  in protein translocation was provided by proteolytic dissection experiments. Treatment of microsomal membranes with a variety of proteases leads to the release of a 52-kD cytoplasmic domain of

SR $\alpha$  from the membrane and a concomitant loss of the ability of these membranes to translocate secretory proteins. Readdition of the purified 52-kD cytoplasmic domain to proteolyzed microsomes will reconstitute functional SRP receptor and restore the translocation activity of the vesicles (22, 31). Recently, we determined the primary sequence of SR $\alpha$  from cDNA clones. We established that SR $\alpha$  is anchored to the ER membrane by its amino-terminal region and that the membrane anchor fragment and the 52-kD cytoplasmic domain jointly contribute to a functionally important region, which is highly charged and may function as the SRP binding site (20). Here we report that the SRP receptor contains an additional subunit of  $\sim$ 30 kD that has not been separated from SR $\alpha$  in previous studies.

## Materials and Methods

### Materials

[<sup>35</sup>S]Methionine (800 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, IL; Na<sup>125</sup>I (100 mCi/ml) from New England Nuclear, Boston, MA; Nikkol (octa-ethylene-mono-*n*-dodecyl ether) from Nikko Chemicals Co., Ltd., Tokyo, Japan; nitrocellulose filters from Schleicher & Schuell, Inc., Keene, NH; Trasylol (10,000 kallikrein inhibition units per ml) from FBA Pharmaceuticals, New York, NY; TPCK-trypsin from Worthington Biochemical Corp., Freehold, NJ; aminopentyl agarose, elastase, papain, chymotrypsin, and protease inhibitors from Sigma Chemical Co., St. Louis, MO; Freund's complete and incomplete adjuvant, anti-mouse Ig and anti-rabbit Ig antibodies from Cappel Laboratories, Malvern, PA;

CNBr-activated Sepharose CL-4B, CM-Sepharose, and protein A-Sepharose from Pharmacia Fine Chemicals, Uppsala, Sweden; Affigel 10, DEAE Affigel Blue, hydroxylapatite from Bio-Rad Laboratories, Richmond, CA.

### **Preparations of Microsomal Membranes, SRP, and Salt-extracted Microsomal Membranes**

These preparations were performed as previously described (28, 29).

### **Preparation of Monoclonal Antibodies to SR $\alpha$**

A 6-wk-old BALB/c mouse was first immunized by an injection into the footpad of 50  $\mu$ g of a 52-kD proteolytic fragment of SR $\alpha$  emulsified with Freund's complete adjuvant. The 52-kD fragment of SR $\alpha$  was purified after elastase digestion of rough microsomal membranes as described (26) and further purified by preparative SDS PAGE (17). Boost immunizations were performed at 2-wk intervals intraperitoneally by injecting 50–100  $\mu$ g of purified SRP receptor (see below) emulsified with incomplete adjuvant. Spleen cells were fused to the myeloma cell line, SP2/o, using polyethylene glycol. The fusion and subsequent selection of hybridomas in hypoxanthine/aminopterin/thymidine medium were performed as described elsewhere (8). Positive clones were detected by Western blotting using alkaline phosphatase-coupled second antibody. The series of cloning gave more than 10 individual clones that recognized two distinct epitopes on SR $\alpha$  (see Results). Subclasses of the monoclonal antibodies were determined using a kit purchased from Boehringer Mannheim Biochemicals, Indianapolis, IN. Hybridoma cells were propagated as ascites tumors. IgG secreted into the ascites fluid was purified on DEAE Affigel Blue (7).

A hybridoma cell line that secretes IgG1, which recognizes a 220-kD cytoskeletal protein, was a generous gift from Dr. David Gard (Department of Biochemistry, University of California at San Francisco).

### **Preparation of Polyclonal Antibodies**

Polyclonal antibodies against SR $\alpha$ , SR $\beta$ , and mp30 were raised in rabbits. SR $\alpha$  and SR $\beta$  were purified by immunoaffinity chromatography and mp30 by affinity chromatography on SRP-Sepharose (see below), followed by preparative SDS PAGE (17). Primary immunization involved 100- $\mu$ g subcutaneous injections of each protein emulsified with Freund's complete adjuvant. For boost immunizations 100- $\mu$ g antigen emulsified with incomplete adjuvant was injected every 2 wk until a serum titer was observed. Anti-SR $\alpha$  and anti-SR $\beta$  antibodies were immunoselected by antigen coupled to Sepharose (24). Anti-mp30 antiserum was generally not immunoselected, but IgG was purified using a DEAE Affigel Blue column.

### **Coupling of Proteins to Gel Matrix**

Each protein fraction, either antigen or antibody, was coupled to CNBr-activated Sepharose CL-4B as described in the Pharmacia manual except that 0.1 M sodium phosphate (pH 6.5) was used as coupling buffer. Anti-SR $\alpha$  monoclonal antibody recognizing epitope A was also coupled to Affigel 10 as described in the Bio-Rad manual.

### **Purification of SRP Receptor**

SRP receptor was purified by two different methods. The first method involved chromatography on aminopentyl agarose, hydroxylapatite, and SRP-Sepharose as described by Gilmore and Blobel (11), with the following modification. During detergent extraction of salt-extracted microsomal membranes, we used additional protease inhibitors (0.1 mM diisopropyl fluorophosphate and 10 U/ml of Trasylol).

For the second method, we used our monoclonal antibody against SR $\alpha$  as affinity adsorbent. Salt- and EDTA-extracted canine pancreatic microsomal membranes were detergent extracted as described above, except that 0.5 mM glutathione was used instead of 1 mM dithiothreitol (DTT) to keep the disulfide bonds of the antibodies intact. In the next step, 80 ml of detergent extract (1,000 eq/ml; 1 eq is the material that is derived from 1  $\mu$ l of rough microsomal membranes at a concentration of 50 A<sub>280</sub> units/ml [28]) was loaded onto 2 ml of IgG-Sepharose (4 mg of monoclonal antibody recognizing epitope A coupled per milliliter of resin). The column was washed with 10 column volumes of 250 mM sucrose, 50 mM triethanolamine-HOAc (pH 7.5), 500 mM potassium acetate (KOAc), 1% Nikkol, and 0.5 mM glutathione. SRP receptor was eluted with 3 column volumes of 4.5 M magnesium chloride, 50 mM triethanolamine-HOAc (pH 7.5), 0.5% Nikkol, and 0.5 mM glutathione. The eluate was dialyzed overnight at 4°C against 1 liter of 250 mM sucrose, 50 mM triethanolamine-HOAc (pH 7.5), and

1 mM DTT. The dialyzed sample was loaded onto 2 ml of CM-Sepharose that had been equilibrated with 250 mM sucrose, 25 mM Hepes-KOH (pH 7.5), 10 mM KOAc, 0.5% Nikkol, and 1 mM DTT. The column was washed with 5 column volumes of the equilibration buffer. SRP receptor was eluted with 2 column volumes of 500 mM KOAc in the same buffer. The SRP receptor preparation was active when assayed for arrest releasing activity. In a quantitative assay half-maximal arrest release was obtained at 9 nM SR $\alpha$  in the presence of 8 nM SRP. This amounts to a similar specific activity as obtained after purification on SRP-Sepharose. As discussed in this paper, SRP receptor is partially dissociating during the purification procedure. According to quantitative measurements, immunopurified SRP receptor samples contained on average 0.46 mol of SR $\beta$  per mol of SR $\alpha$ .

### **Immunoblotting**

Immunoblotting was done as described by Fisher et al. (9) with the following exceptions. To detect the primary antibody, 100,000 cpm/lane of <sup>125</sup>I-labeled second antibody was used. Secondary antibodies were labeled using chloramine T (6). Whenever monoclonal antibodies were used as primary antibodies, SDS was excluded from all of the buffers. For the quantitation of the specific protein bands, the immunoblotted nitrocellulose was cut and radioactivity in the bands was determined in a Beckman gamma well scintillation counter.

### **Assays for Arrest Releasing Activity of SRP Receptor**

These assays were performed as previously described (26).

### **Quantitation of SR $\alpha$ and SR $\beta$**

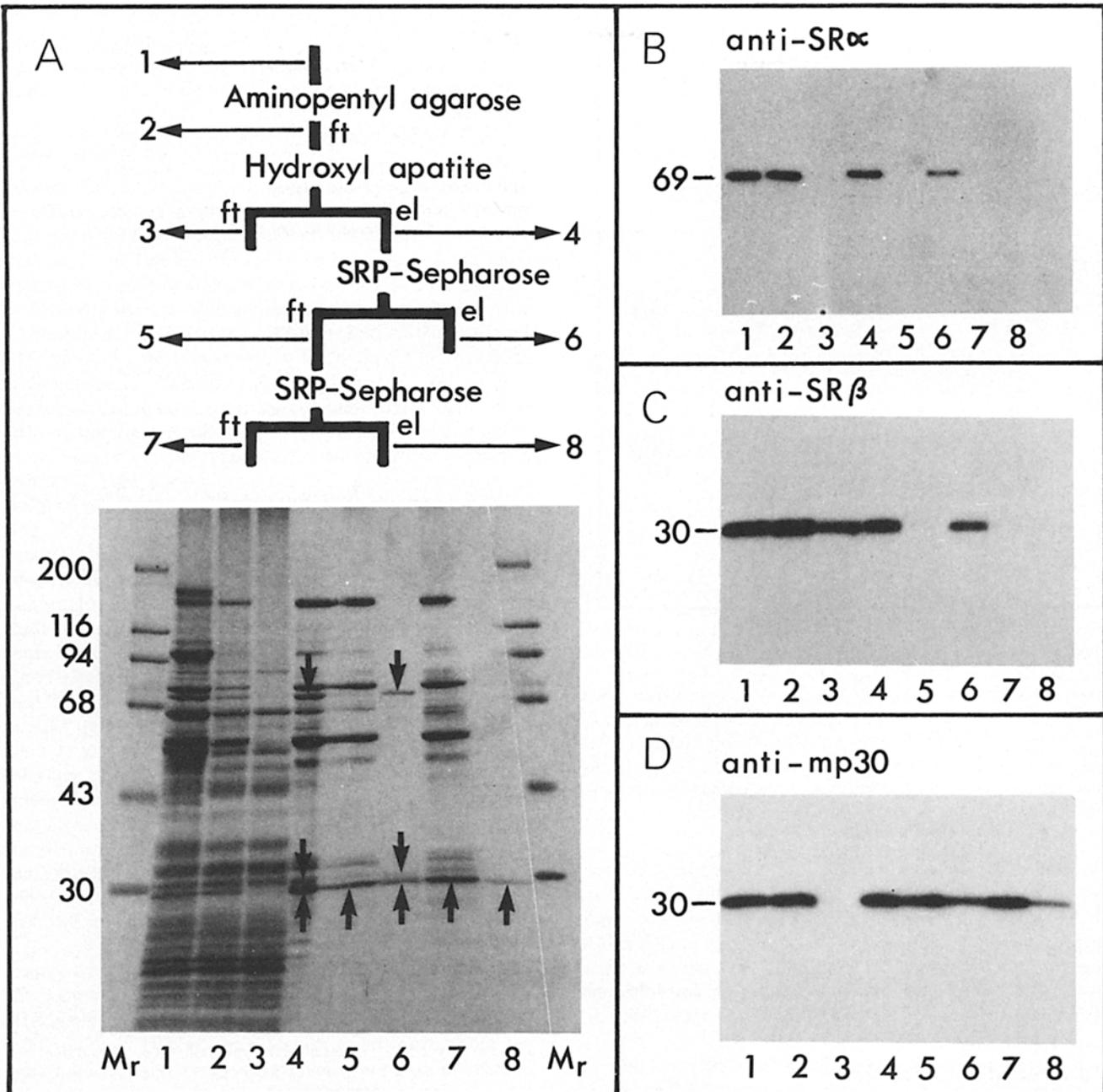
SR $\alpha$  and SR $\beta$  that had been electroeluted from preparative gels by SDS PAGE were quantitated by amino acid analysis. The proteins were hydrolyzed in 6 N HCl in the presence of phenol in vacuo at 108°C for 24 h. Phenylisothiocyanate-derivatized amino acids were analyzed and quantitated by high performance liquid chromatography as described (16). Under the given hydrolysis condition cysteine and tryptophan residues are degraded. Also, a slight loss of threonine and serine residues may have occurred. We estimate that these degradations effect the quantitation of SR $\beta$  to <10%. For SR $\alpha$  the primary sequence is known from cDNA cloning and verifies this assumption.

### **Subfractionation of Rat Liver Microsomes**

Subfractionation was performed according to the method of Beaufay et al. (3). Female Wistar rats that weighed 150–200 g were starved for 18 h. Rats were killed and the livers removed. A liver that weighed 6 g was homogenized in 54 ml of 0.25 M sucrose in 10 mM Tris-HCl (pH 7.5) with four strokes in a motor-driven Teflon homogenizer. The homogenate was centrifuged at 3,000 rpm (1,000  $g_{av}$ ) for 10 min in a Beckman J-21 rotor (Beckman Instruments, Inc., Palo Alto, CA), and floated fat was removed. The supernatant fraction was then centrifuged again at 10,000 rpm (10,000  $g_{av}$ ) for 10 min in the same rotor. The supernatant fraction was centrifuged in a Beckman type 50.2 Ti rotor at 40,000 rpm (140,000  $g_{av}$ ) for 60 min. The pellet was suspended in 25 ml of homogenizing buffer and repelleted as above. The final pellet was resuspended in 6 ml of homogenization buffer. An aliquot of this microsomal fraction (1 ml) was loaded onto a 12 ml sucrose gradient ( $d = 1.100$ – $1.250$  g/ml) containing a 0.5 ml sucrose cushion ( $d = 1.34$  g/ml), and centrifuged at 4°C in a Beckman SW 40 rotor at 40,000 rpm (200,000  $g_{av}$ ) for 20 h. The gradient was fractionated into 0.8 ml per tube. Sucrose concentrations were determined by refractometry (Atago), protein concentration was determined by the method of Schaffner and Weissman (25), RNA by Fleck and Munro (10), and phospholipid phosphorus by the method of Ames and Dubin (2) after extraction of phospholipid by chloroform/methanol (5). SR $\alpha$ , SR $\beta$ , and ribophorin II were determined by Western blotting using <sup>125</sup>I-labeled secondary antibodies. Monoclonal antibodies recognizing ribophorin II were generous gifts of Dr. Gert Kreibich (New York University). NADPH cytochrome *c* reductase activity was determined as described (4).

### **Results**

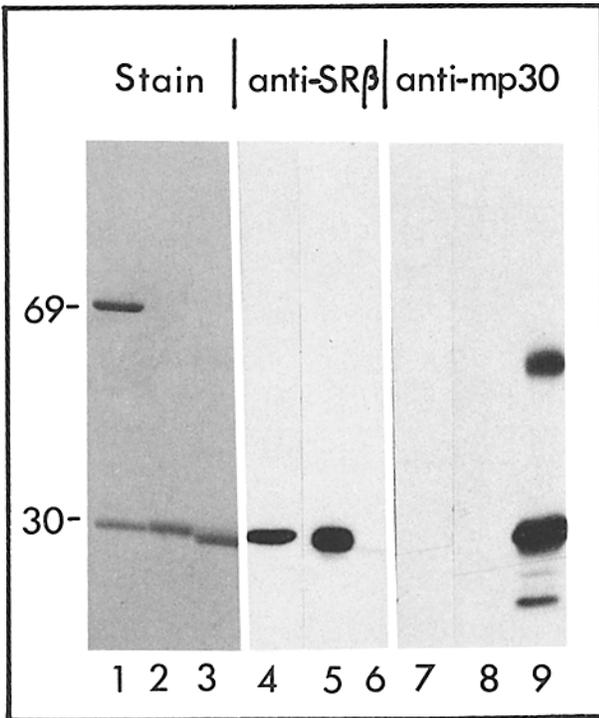
Chromatography on SRP-Sepharose as a key purification step was used by Gilmore et al. (12) as the original purification procedure for SRP receptor. When we purified SRP



**Figure 1.** Purification of SRP receptor on SRP-Sepharose. Key fractions of the purification procedure (outlined schematically in *A*) were subjected to electrophoresis on 10–15% SDS polyacrylamide gels. The following samples were loaded: lanes 1, detergent extract of salt-extracted microsomes that was loaded onto aminopentyl agarose; lanes 2, flow-through fraction from aminopentyl agarose that was loaded onto hydroxylapatite; lanes 3, flow-through fraction from hydroxylapatite that was loaded onto SRP-Sepharose; lanes 4, eluate from hydroxylapatite that was loaded onto SRP-Sepharose; lanes 5, flow-through fraction from SRP-Sepharose. This fraction was re-loaded onto a fresh SRP-Sepharose column (see lanes 7 and 8). Lanes 6, eluate from SRP-Sepharose; lanes 7, flow-through fraction from SRP-Sepharose after reloading of the first flow-through fraction shown in lanes 5; lanes 8, eluate from SRP-Sepharose after reloading of the first flow-through fraction shown in lanes 5. All samples were loaded at 10 eq, except lanes 4–8 in *A*, where the load was increased to 50 eq. (*A*) Coomassie Blue-stained SDS gel. Downward arrows indicate SR $\alpha$  (upper) and SR $\beta$  (lower), respectively. Upward arrows indicate mp30. (*B*) The SDS gel was blotted onto nitrocellulose and probed with immunoselected rabbit polyclonal anti-SR $\alpha$  Ig. Bound Ig was detected after incubation with a <sup>125</sup>I-labeled secondary antibody and autoradiography (see Materials and Methods). (*C*) Same as *B*, but immunoselected polyclonal anti-SR $\beta$  was used to probe the blot. (*D*) Same as *B*, but polyclonal anti-mp30 IgG was used to probe the blot. Only the relevant sections of the blots are shown in *B*–*D*. Regions above and below the shown sections were blank.

receptor from a detergent extract of canine microsomal membranes, we noticed the purification of other polypeptides in addition to the previously described and sequenced 69-kD polypeptide (SR $\alpha$ ). Fig. 1 *A* shows the polypeptide

profiles of the key fractions in the purification procedure. Elution from SRP-Sepharose (Fig. 1 *A*, lane 6) reproducibly yielded SR $\alpha$  (upper downward arrow) as well as two prominent, closely spaced bands with a molecular mass of ~30 kD



**Figure 2.** Characterization of antibodies to SR $\beta$  and mp30. SRP receptor purified by immunoaffinity chromatography (see Materials and Methods and Fig. 3) (lanes 1, 4, and 7), SR $\beta$  purified by preparative SDS PAGE (see Materials and Methods) (lanes 2, 5, and 8), and mp30 purified by preparative SDS PAGE (lanes 3, 6, and 9) were displayed on a 10–15% SDS polyacrylamide gel. The gel was either stained with Coomassie Blue (lanes 1–3) or blotted onto nitrocellulose and probed with immunoselected rabbit anti-SR $\beta$  (lanes 4–6) or anti-mp30 (lanes 7–9) (see Fig. 1).

(lower arrows) and a number of minor polypeptides. For reasons that are discussed below we refer to the upper, more fuzzy 30-kD band (Fig. 1 A, lanes 4 and 6, lower downward arrows) as the  $\beta$ -subunit of the SRP receptor (SR $\beta$ ) and to the sharp lower band (Fig. 1 A, lanes 4–8, upward arrows) as mp30. None of the proteins bound to control Sepharose columns that contained immobilized bovine serum albumin (data not shown).

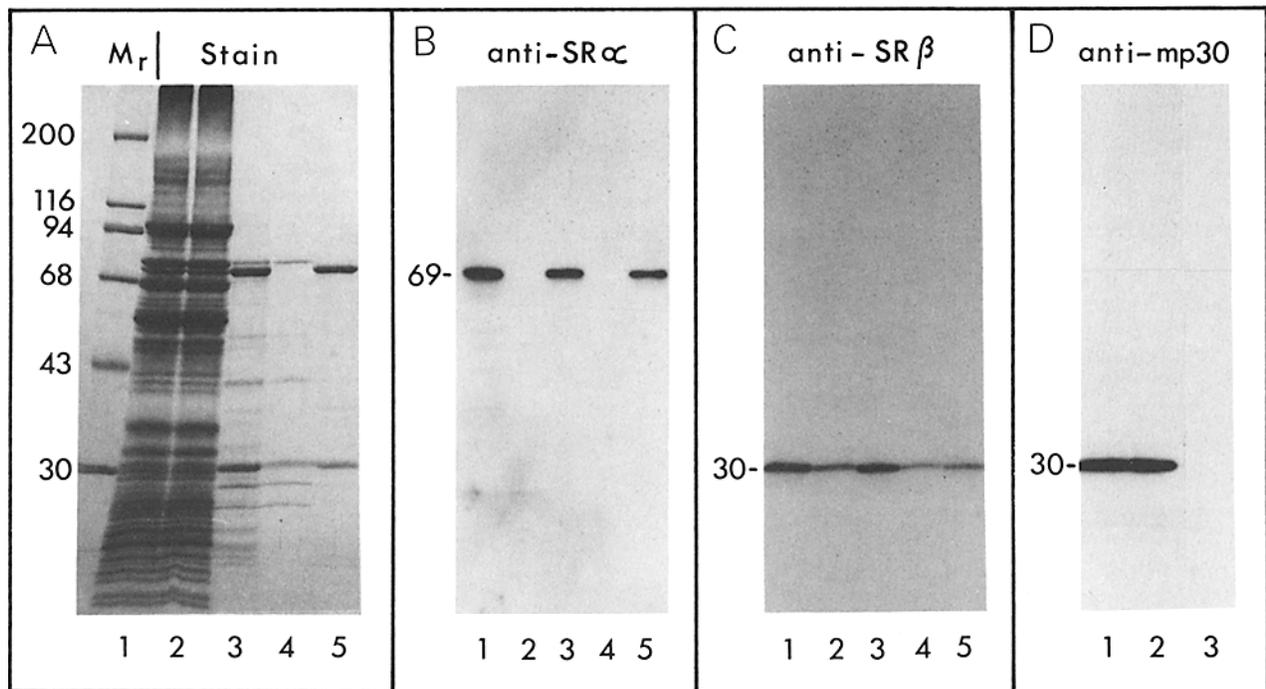
To further investigate the nature of these polypeptides, we raised in rabbits polyclonal antibodies against SR $\beta$  and mp30 after purification of the denatured antigens by preparative SDS PAGE (see Materials and Methods). Fig. 2 shows a characterization of the obtained antisera. SR $\beta$  (Fig. 2, lanes 2, 5, and 8) and mp30 (Fig. 2, lanes 3, 6, and 9) were purified by preparative SDS PAGE, re-electrophoresed, and blotted onto nitrocellulose. SRP receptor (purified on anti-SR $\alpha$  Sepharose and therefore devoid of mp30, see below) is analyzed in lanes 1, 4, and 7. Lanes 1–3 show the Coomassie Blue-stained gel before blotting, lanes 4–6 the blot probed with anti-SR $\beta$ , and lanes 7–9 the blot probed with anti-mp30. It is apparent from these data that anti-SR $\beta$  does not recognize mp30 (compare Fig. 2, lanes 4 and 5 with lane 6) and anti-mp30 is also not cross-reactive with SR $\beta$  (compare Fig. 2, lane 9 with lanes 7 and 8). The higher molecular weight band in lane 9 is apparently a dimer of mp30 that is not dissociated in SDS. This irreversible oligomerization (oligomers up to penta- and hexamers of mp30 are detectable by stain and immunoblot in overloaded gels) was induced

during purification by preparative SDS PAGE and is not detectable if the eluate of the SRP-Sepharose is analyzed directly (see Fig. 1 D, lanes 6 and 8). Note that neither anti-SR $\beta$  (Fig. 2, lane 4) nor anti-mp30 (Fig. 2, lane 7) recognizes SR $\alpha$ .

The availability of specific antibodies allowed us to detect and estimate the amounts of SR $\alpha$  (Fig. 1 B), SR $\beta$  (Fig. 1 C), and mp30 (Fig. 1 D) in various fractions of the SRP receptor purification procedure. All three proteins fractionate like ER membrane proteins in the earlier steps of the purification. They are not extracted by EDTA or high salt but require detergents to be solubilized (data not shown). The detergent extract was fractionated on aminopentyl agarose followed by hydroxylapatite. Note that SR $\alpha$  and mp30 are quantitatively recovered in the eluate of the hydroxylapatite column (Fig. 1, B and D, lanes 4), whereas some SR $\beta$  (about one-third of the load) is also found in the flow-through fraction of this column (Fig. 1 C, lane 3).<sup>2</sup> Upon chromatography on SRP-Sepharose we found that SR $\beta$  is quantitatively retained by the affinity column (Fig. 1 C, compare lanes 4 and 5) and then co-eluted with SR $\alpha$  (Fig. 1, B and C, lanes 6). In contrast, only a fraction of mp30 binds to SRP-Sepharose and the bulk is recovered in the flow-through fraction (Fig. 1 D, lanes 4–6). When the flow-through fraction (Fig. 1, A and D, lanes 5) was re-applied to a fresh SRP-Sepharose column, an equivalent amount of mp30 bound to the column and could be eluted (Fig. 1, A and D, lanes 8). A large amount of mp30 was still recovered in the flow-through of this second passage (Fig. 1, A and D, lanes 7). Thus we conclude that mp30 interacts with SRP-Sepharose directly and not through SR $\alpha$  or SR $\beta$ , because binding occurs even in the absence of these two proteins. When an SRP-Sepharose column was saturated with the load fraction, we found that the affinity column binds approximately equal molar amounts of SR $\alpha$ , SR $\beta$ , and mp30. From the Coomassie staining intensity of mp30 and from its distribution into various fractions during the purification, we estimate that mp30 is present in microsomal membranes in roughly 5–20-fold molar excess of SR $\alpha$  and SR $\beta$  (see below).

Purification of SRP receptor by a different strategy provided us with direct evidence that SR $\alpha$  and SR $\beta$  interact with each other. Fig. 3 A shows a Coomassie Blue-stained SDS gel monitoring the purification of the SRP receptor on a Sepharose column containing a covalently bound mono-

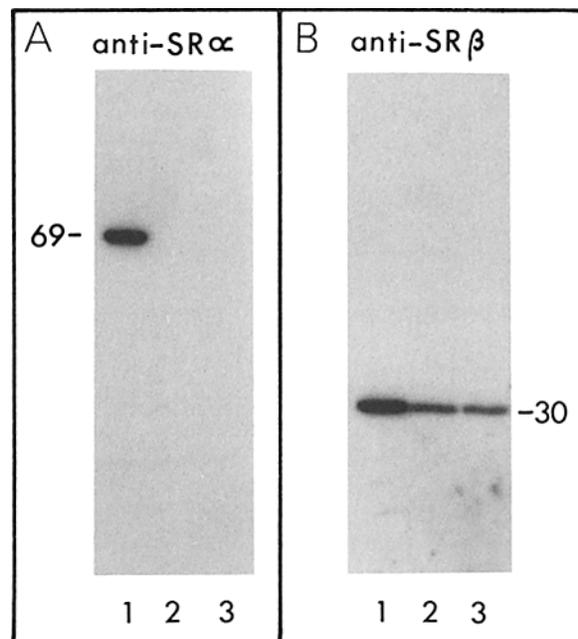
2. Partial dissociation of SR $\alpha$  and SR $\beta$  was observed upon chromatography on hydroxylapatite (Fig. 1, B and C, lanes 3), CM-Sepharose (Fig. 3, B and C, lanes 4), and upon immunopurification with antibodies to SR $\alpha$  (Fig. 3, B and C, lanes 2) and SR $\beta$  (Fig. 8, B and C). A rough estimate shows that about one-third of SR $\beta$  dissociates from SR $\alpha$  in each of the described chromatography steps in Figs. 1 and 3 (with the notable exception of SRP Sepharose, see below). Almost 80% of SR $\alpha$  was dissociated when SRP receptor was immunoprecipitated with polyclonal anti-SR $\beta$  (Fig. 8, B and C). We interpret this finding to mean that during these fractionations some SR $\alpha$  and SR $\beta$  are dissociating due to an irreversible denaturation of either protein that is induced by its interaction with the chromatography resins or antibodies. The dissociations are only induced during the binding steps of SRP receptor; no further dissociation was observed upon subsequent washing steps. This indicates that the observed effects are not due to an intrinsically weak affinity of the two subunits for each other. Similar "induced dissociations" have been observed previously, e.g., for the E1 and E2 coat proteins of Semliki Forest virus, where both proteins clearly exist as a heterodimer, yet fail to be immunoprecipitable as such (32). Note that no dissociation was observed when SRP receptor was chromatographed on SRP-Sepharose (Fig. 1, B and C, lanes 5). On this column SRP receptor is retained presumably through a functional interaction that does not perturb SRP receptor structure.



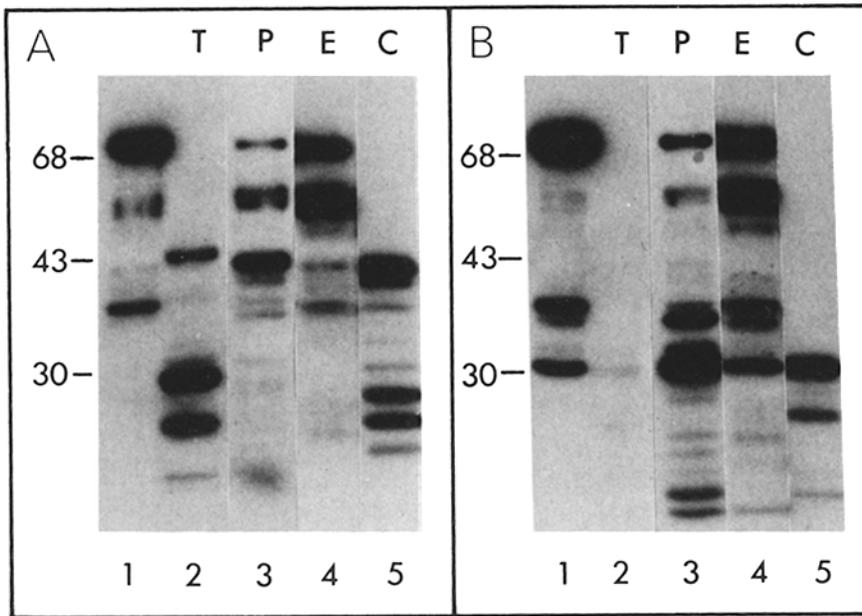
**Figure 3.** Purification of SRP receptor by immunoaffinity chromatography. Fractions of the purification procedure as detailed in Materials and Methods are shown. A monoclonal antibody recognizing epitope A was coupled to Sepharose. Lanes 1, detergent extract of salt-extracted microsomes that was loaded onto the antibody column; lanes 2, flow-through fractions of the immunoaffinity column; lanes 3, eluate from the immunoaffinity column that was loaded onto CM-Sepharose; lanes 4, flow-through fractions of the CM-Sepharose column; lanes 5, eluate from the CM-Sepharose column. Fractions were analyzed by SDS PAGE after Coomassie Blue staining (A) or immunoblotting with polyclonal antibodies to SR $\alpha$  (B), SR $\beta$  (C), or mp30 (D). All the lanes were loaded with 10 eq, except lanes 3–5 in A, where the load was increased to 100 eq.

clonal antibody raised against SR $\alpha$ . A detergent extract of microsomal membranes (Fig. 3 A, lane 1) was passed over the IgG-Sepharose column. The column was washed and then eluted with 4.5 M magnesium chloride, a mild chaotrope. SR $\alpha$  and SR $\beta$  are the major polypeptides visible in the eluate (Fig. 3 A, lane 3). SR $\alpha$  was quantitated by Western blot analysis (Fig. 3 B) and found quantitatively recovered (Fig. 3 B, compare lanes 1 and 3). Minor contaminants (including some IgG light chain eluting from the column) are removed by subsequent chromatography on CM-Sepharose (Fig. 3, lanes 3–5). The eluted SRP receptor is active when assayed for arrest releasing activity. To what extent the partial loss of SR $\beta$  (see below) leads to a reduced specific activity of SRP receptor remains to be determined.

When SRP receptor was purified on the immunoaffinity and CM-Sepharose columns, we again observed the co-fractionation of a 30-kD polypeptide with SR $\alpha$  (Fig. 3 A, lane 5). Western blot analysis with either anti-SR $\beta$  (Fig. 3 C) or anti-mp30 (Fig. 3 D) shows that SR $\beta$  co-purified with SR $\alpha$  (Fig. 3 C, lane 5), whereas mp30 was quantitatively recovered in the flow-through fraction of the IgG-Sepharose column (Fig. 3 D, lane 2). Note that SR $\beta$  can also be detected in the flow-through fraction of both the IgG-Sepharose column (Fig. 3 C, lane 2) and the CM-Sepharose column (Fig. 3 C, lane 4).<sup>2</sup> The dissociation occurs during the binding steps of SRP receptor to the chromatography resins. During subsequent washing steps no further SR $\beta$  was removed (data not shown), i.e., once intact receptor is bound it is stable. Under these conditions, the dissociation of SR $\beta$  was irreversible, because dissociated SR $\beta$  in the flow-through fraction could not bind to the IgG-Sepharose when



**Figure 4.** Binding behavior of dissociated SR $\beta$  to anti-SR $\alpha$ -Sepharose. A detergent extract of salt-extracted microsomes was passed over an immunoaffinity column as described in Fig. 3 at a constant flow rate of 8 ml/h. The flow-through fraction was reloaded onto the same column at the same flow rate. Samples (10 eq) of load (lanes 1), first flow-through fraction (lanes 2), and second flow-through fraction (lanes 3) were resolved by SDS PAGE and immunoblotted with anti-SR $\alpha$  (A) or anti-SR $\beta$  (B). Note that no additional SR $\beta$  was retained upon the second passage through the column.



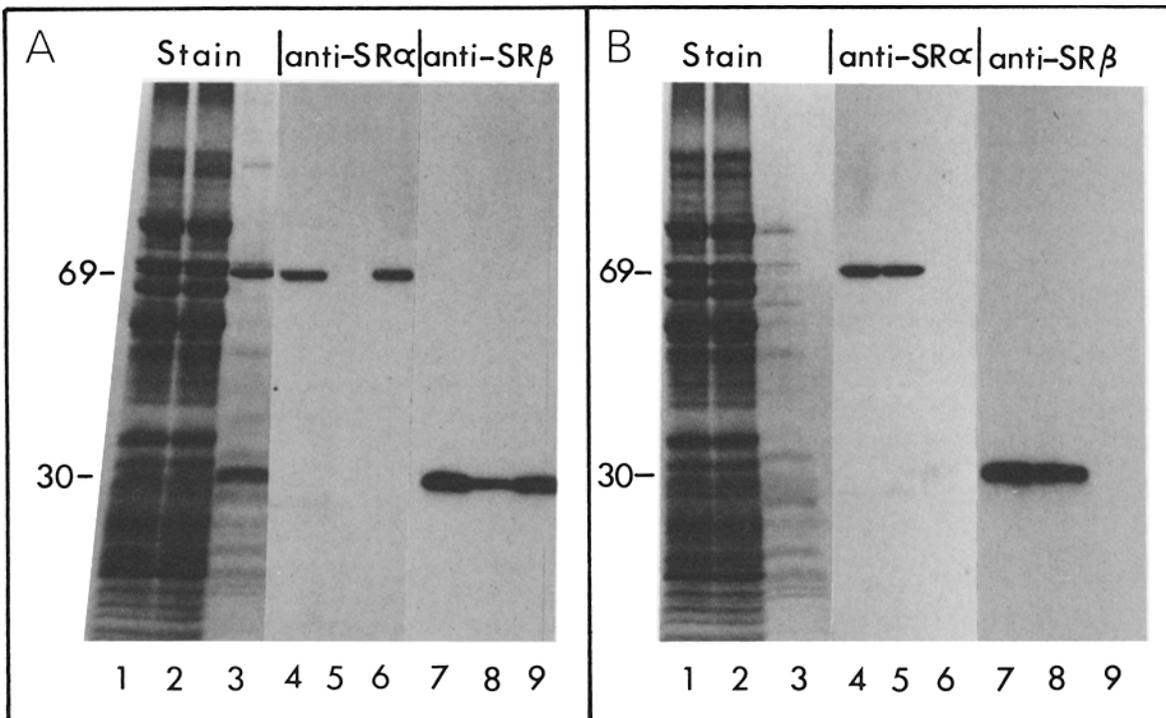
**Figure 5.** One-dimensional peptide mapping of SR $\alpha$ . SRP receptor (150  $\mu$ g/ml, prepared by immunoaffinity chromatography followed by CM-Sepharose chromatography), was digested with TPCK-trypsin (T, lanes 2), papain in the presence of 30 mM cysteine (P, lanes 3), elastase (E, lanes 4), chymotrypsin (C, lanes 5), or no proteases (lanes 1). All proteases were added to 1:500 (wt/wt), except for elastase which was added to 1:100 (wt/wt). Digestions were for 1 h at 37°C in the elution buffer of the CM-Sepharose column (see Materials and Methods). Digests derived from 0.5  $\mu$ g of SRP receptor were displayed by SDS PAGE and blotted onto nitrocellulose. Blots were probed with monoclonal antibodies recognizing epitope A (A) or epitope B (B) on SR $\alpha$ .

re-applied to the same column (Fig. 4 B, compare lanes 2 and 3).

To eliminate the possibility that SR $\beta$ , rather than interacting with SR $\alpha$ , somehow interacted with the quenched CNBr-activated Sepharose or the particular IgG1, we performed the following control experiments. First, anti-SR $\alpha$  was coupled

to a different matrix (Affi Gel 10) and used as the affinity absorbent. We found that the elution behaviors of SR $\alpha$  and SR $\beta$  were indistinguishable from that shown in Fig. 3 (data not shown), indicating that SR $\beta$  is not bound due to interaction with the gel matrix.

Second, different monoclonal antibodies were used as the

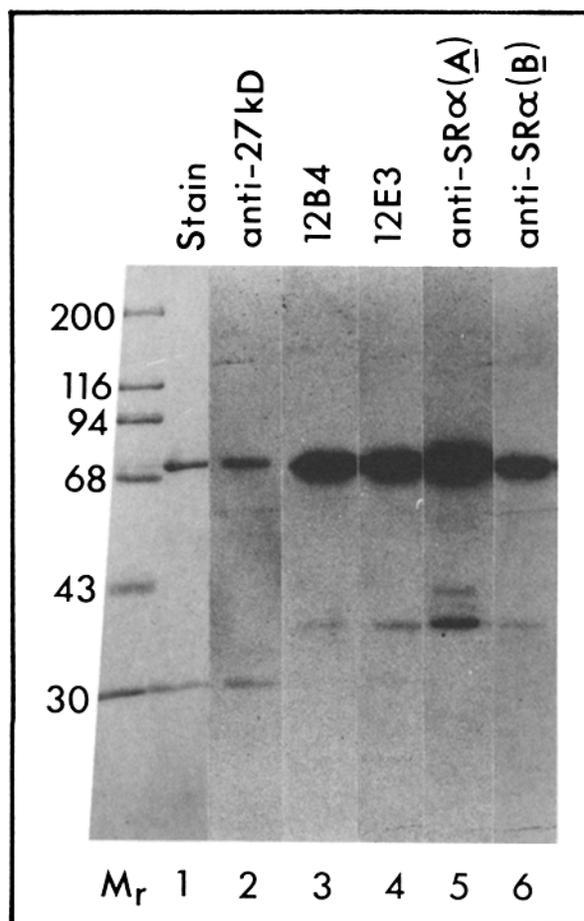


**Figure 6.** Chromatography of SRP receptor on control immunoaffinity columns. Immunoaffinity columns were prepared by either coupling a monoclonal IgG1 recognizing epitope B on SR $\alpha$  (A) or a control IgG1 recognizing a 220-kD cytoskeletal protein (a gift of Dr. D. Gard) (B) to CNBr-activated Sepharose. Coupling densities were 2 and 5 mg per ml of resin, respectively. A detergent extract of microsomal vesicles was loaded onto the columns and eluted as described in Fig. 3. Samples were displayed by SDS PAGE and Coomassie Blue staining (lanes 1–3), or immunoblotting with anti-SR $\alpha$  (lanes 4–6) or anti-SR $\beta$  (lanes 7–9) (see Fig. 1). Lanes 1, 4, and 7 show the detergent extract, lanes 2, 5, and 8 show the flow-through fractions, and lanes 3, 6, and 9 show the column eluates. Each lane was loaded with 10 eq, except lanes 3 which were loaded with 100 eq.

affinity adsorbent for SRP receptor. 10 different hybridoma cell lines producing monoclonal antibodies to SR $\alpha$  were originally isolated. We could group these monoclonal antibodies into two distinct groups that recognize different epitopes (epitope *A* and epitope *B*) on SR $\alpha$  using a one-dimensional peptide mapping approach. Purified SRP receptor (Fig. 3, lane 5) was subjected to limited proteolysis using a variety of different proteases. Fragments were fractionated by SDS PAGE and blotted onto nitrocellulose. Very discrete and characteristic patterns were obtained when the blot was probed with either anti-SR $\alpha$  recognizing epitope *A* (Fig. 5 *A*) or anti-SR $\alpha$  recognizing epitope *B* (Fig. 5 *B*). Thus, both epitopes mark physically separate locations on the SR $\alpha$  polypeptide. In the immunopurification of SRP receptor shown in Fig. 3, an IgG1 recognizing epitope *A* was used. When we repeated the experiment using an IgG1 recognizing epitope *B*, identical results were obtained (Fig. 6 *A*). As expected, if a control IgG1 Sepharose column was used, neither SR $\alpha$  nor SR $\beta$  bound to the column (Fig. 6 *B*).

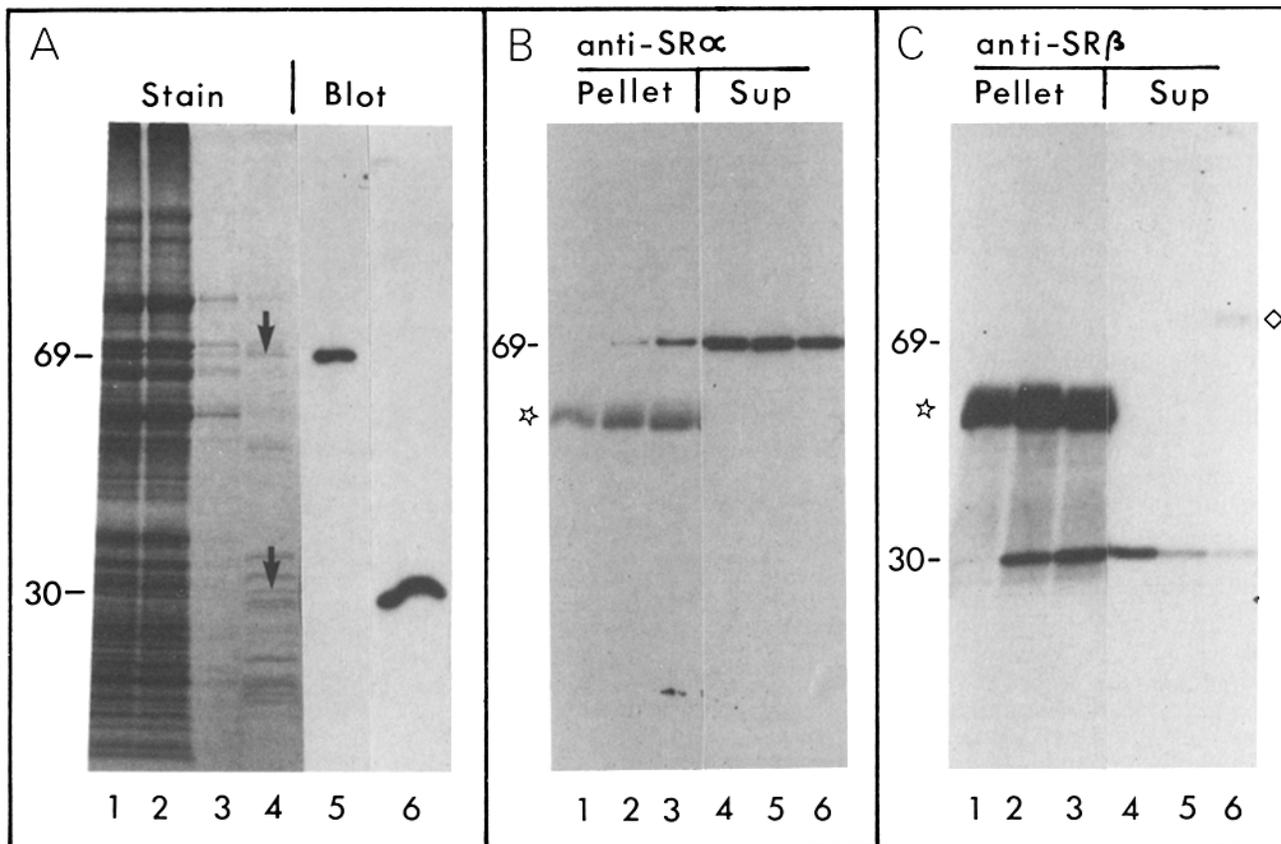
Previously, Hortsch et al. also reported a method using a monoclonal antibody as affinity probe for the purification of SR $\alpha$  (14). In addition to SR $\alpha$  these investigators also found a peptide of  $M_r$  27,000 in their column eluate, but concluded that the peptide was a degradation product of SR $\alpha$ . This conclusion was based on their observation that a band of  $\sim$ 27 kD was recognized by their monoclonal antibodies to SR $\alpha$  (12B4 and 12E3). Furthermore, Hortsch et al. raised a polyclonal rabbit antibody against this 27-kD polypeptide and found it cross-reactive to SR $\alpha$ . We have obtained samples of their antibodies and used them to probe our SRP receptor preparation (Fig. 7, lane 1). We conclude that: (a) There is no cross-reacting material in the 30-kD range when the blot is probed with 12B4 or 12E3 (Fig. 7, lanes 3 and 4), i.e., SR $\beta$  is not detected by these antibodies. The visible minor breakdown product of SR $\alpha$  migrates at  $\sim$ 40 kD. (b) The polyclonal anti-27-kD serum recognizes both SR $\alpha$  and SR $\beta$  (Fig. 7, lane 2). This indicates that breakdown product(s) of SR $\alpha$  must have been present in the 30-kD range during antigen preparation by Hortsch et al., which then elicited the observed immune response against SR $\alpha$ . These data also demonstrate that SR $\beta$  is present in the SRP receptor preparations of Hortsch et al., since their anti-27-kD serum recognizes it (Fig. 7, lane 2). Occasionally, we also observed a minor 30-kD breakdown product of SR $\alpha$  in SRP receptor preparations (e.g., Fig. 5 *B*, lane 1). This degradation was prevented in subsequent preparations by the inclusion of protease inhibitors during the detergent extraction (see Materials and Methods; Fig. 7).

The data presented so far demonstrate that SR $\alpha$  and SR $\beta$  reproducibly co-purify by different isolation procedures. If both polypeptides exist as a complex, we can expect also to immunopurify SR $\alpha$  with anti-SR $\beta$ . Fig. 8 shows the results from such an experiment. Rabbit anti-SR $\beta$  Ig was prepared by immunopurification (see Materials and Methods). The Ig fraction was coupled to CNBr-activated Sepharose. A detergent extract from microsomal membranes was then passed over the immunoaffinity column (Fig. 8 *A*, lanes 1 and 2). The column was washed (Fig. 8 *A*, lane 3) and then eluted with 4.5 M magnesium chloride (Fig. 8 *A*, lane 4). SR $\alpha$  (Fig. 8 *A*, lane 5) and SR $\beta$  (Fig. 8 *A*, lane 6), but no mp30 (not shown), were detected in the eluate, i.e., both subunits of SRP receptor also co-purify by this technique.



**Figure 7.** Immunoblot analysis of various antisera and antibodies. Immunopurified SRP receptor (0.5  $\mu$ g SR $\alpha$  per lane) was subjected to SDS PAGE and stained with Coomassie Blue (lane 1) or immunoblotted (see Fig. 1) with the following antibodies: lane 2, polyclonal rabbit antiserum raised against a 27-kD protein (14), lanes 3 and 4, monoclonal antibodies 12B4 and 12E3 (14), and lanes 5 and 6, monoclonal antibodies described in this paper recognizing epitope *A* or epitope *B* on SR $\alpha$ , respectively. Anti-27-kD serum, 12B4, and 12E3 ascites fluids were obtained from Drs. M. Hortsch and D. Meyer.

The result presented in Fig. 8 *A* is of a qualitative nature. We performed a series of immunoadsorption experiments attempting to quantitate the amount of SR $\alpha$  that can be co-immunoprecipitated by anti-SR $\beta$ . In Fig. 8 *B* and *C* a typical experiment is shown. Anti-SR $\beta$  was titrated into an extract of microsomal membranes, immune complexes were precipitated with protein A-Sepharose, and both the pellet (Fig. 8, *B* and *C*, lanes 1-3) and the supernatant fractions (Fig. 8, *B* and *C*, lanes 4 and 5) were analyzed by SDS PAGE followed by Western blotting. Blots were either probed with anti-SR $\alpha$  (Fig. 8 *B*) or anti-SR $\beta$  (Fig. 8 *C*). As expected, we depleted the supernatant fractions of SR $\beta$  with increasing antibody concentrations (Fig. 8 *C*, lanes 4-6) and recovered SR $\beta$  in the corresponding pellet fractions (Fig. 8 *C*, lanes 1-3). However, SR $\alpha$  was only incompletely precipitated (Fig. 8 *B*, compare lane 3 with lane 6), even at the higher antibody concentrations where SR $\beta$  was largely depleted from the extracts. We estimate that only  $\sim$ 20% of SR $\alpha$  is immunoprecipitable with anti-SR $\beta$ . We interpret these results that anti-SR $\beta$  (which is a polyclonal antibody and therefore pre-



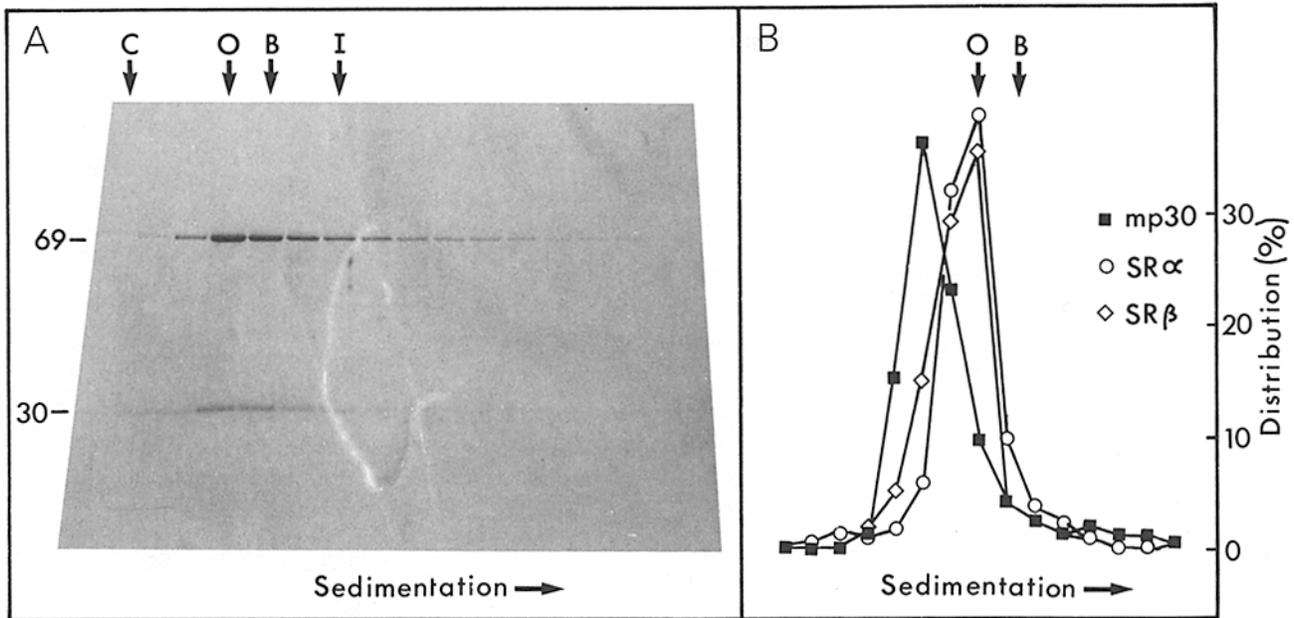
**Figure 8** Co-fractionation of SR $\alpha$  by immunoadsorption on anti-SR $\beta$ . (A) An immunoaffinity column was prepared by coupling 0.5 mg of immunoselected polyclonal rabbit anti-SR $\beta$  to 0.8 ml of CNBr-activated Sepharose. A detergent extract of microsomes (9,000 eq) was chromatographed on this column as described in Fig. 3. Fractions were subjected to SDS PAGE and stained with Coomassie Blue (lanes 1–4). Lane 1 shows the load, lane 2 the flow-through fraction, lane 3 the wash fraction, and lane 4 the eluate of the immunoaffinity column. SRP receptor was detected in the eluate fraction by immunoblot analysis with anti-SR $\alpha$  (lane 5) or anti-SR $\beta$  (lane 6). Samples of 10 eq were loaded in lanes 1 and 2, and 100 eq in lanes 3–6. Both SR $\alpha$  and SR $\beta$  were also detected in the flow-through fraction (not shown), indicating that the column was saturated under the given conditions. Also, the eluate fraction consistently contained other polypeptides unrelated to the SRP receptor subunits (lane 4, SR $\alpha$  and SR $\beta$  are indicated by arrows). This may be a result of the low coupling density of the antibodies on the resin and/or the use of a polyclonal Ig fraction. (B and C) Immunoprecipitations of SRP receptor from a microsomal detergent extract. Increasing amounts of immunoselected polyclonal rabbit anti-SR $\beta$  (no Ig [lanes 1 and 4], 10  $\mu$ g Ig [lanes 2 and 5], and 23  $\mu$ g Ig [lanes 3 and 6]), were incubated with 6 eq of detergent extract (final volume 100  $\mu$ l) at room temperature for 6 h. A 20- $\mu$ l aliquot of protein A-Sepharose was added. The gel matrix was pelleted and washed once with a phosphate-buffered saline solution containing 0.1% Nikkol detergent. Pellets (lanes 1–3) and combined wash and supernatant fractions (lanes 4–6) were subjected to SDS PAGE and blotted onto nitrocellulose. Blots were probed with anti-SR $\alpha$  (B) or anti-SR $\beta$  (C), respectively. The positions of SR $\alpha$  and SR $\beta$  are indicated by their respective molecular weights. The heavy bands marked with an asterisk that are present in the pellet fractions correspond to protein A that has leaked off the Sepharose resin during SDS PAGE sample preparation and that binds antibodies on the nitrocellulose blot. IgG heavy chain migrates in the same position and may contribute to the signal in the lanes where antibody was added. Another minor band (indicated with a diamond in C, lanes 5 and 6) corresponds to the IgM heavy chain. This was shown by probing an equivalent blot with an anti-IgM specific secondary antibody (not shown). As expected, this band is found in the supernatant fraction only, since IgM does not bind to protein A. Both the asterisk and diamond bands are less pronounced in B, because the blot was probed with a mouse monoclonal IgG1 and a secondary anti-mouse IgG antibody.

sumably binds to multiple epitopes on SR $\beta$ ) causes a similar dissociation of the two subunits as we also observed—although to a lesser extent—for anti-SR $\alpha$ .<sup>2</sup>

Given the complication of incomplete immunoprecipitations it became important to demonstrate that SR $\alpha$  and SR $\beta$  form a complex when their structures were not perturbed by bound antibodies. We therefore subjected purified SRP receptor (Fig. 9 A) or a detergent extract of microsomal membranes (Fig. 9 B) to velocity sedimentation analysis. Under both conditions SR $\alpha$  and SR $\beta$  co-sedimented almost indistinguishably from one another, but clearly off-set from mp30 (Fig. 9 B). The peaks that we obtained when crude extracts

were centrifuged (Fig. 9 B) were always sharper than those obtained from purified SRP receptor (Fig. 9 A), indicating that some aggregation may have occurred in the purified sample. Comparison of SRP receptor with sedimentation marker proteins showed that SRP receptor sediments with a velocity similar to that of ovalbumin ( $S = 3.7$ ). Given that ovalbumin (43 kD) is only half the size of SRP receptor (SR $\alpha$  + SR $\beta$  = 100 kD), this anomalous sedimentation must be due to effects caused by bound detergent and/or an extended structure of the SRP receptor that deviates from that of a spherical particle.

Next we determined the absolute amounts of SR $\alpha$  and SR $\beta$



**Figure 9.** Sedimentation analysis of SRP receptor. (A) Immunopurified SRP receptor (10  $\mu$ g) was loaded onto a 13–28% sucrose gradient (5 ml) containing 500 mM KOAc, 5 mM magnesium acetate, 50 mM triethanolamine-HOAc (pH 7.5), 0.5% Nikkol, and 1 mM dithiothreitol, and centrifuged for 18 h at 48,000 rpm (210,000  $g_{av}$ ) in a Beckman SW50.1 swinging bucket rotor. Eight-drop fractions were collected from the bottom of the tube after puncturing. Fractions were subjected to SDS PAGE and stained in Coomassie Blue. The migration of marker proteins in parallel gradients is indicated: C, cytochrome *c* (2.1S); O, ovalbumin (3.7S); B, bovine serum albumin (4.3S); and I, rabbit IgG (7.1S). (B) A microsomal detergent extract (100 eq) was fractionated as described for A, except that a 5–20% sucrose gradient was used. Fractions were subjected to SDS PAGE, blotted onto nitrocellulose, and probed with anti-SR $\alpha$  (○), anti-SR $\beta$  (◇), and anti-mp30 (■). Bands were quantitated by counting the radioactivity of the bound secondary antibody. The total radioactivity for each polypeptide was normalized to 100. Markers are as in A.

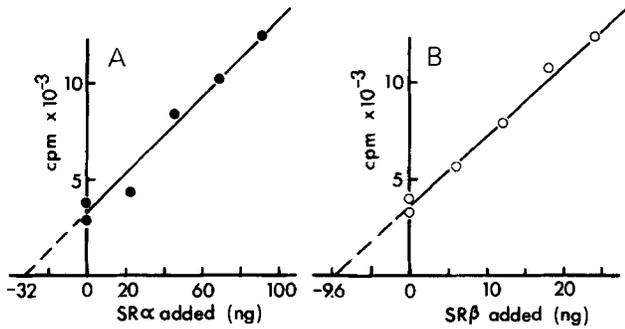
and their relative stoichiometry in microsomal membranes. SR $\alpha$  and SR $\beta$  were separated by preparative SDS PAGE, electroeluted from the SDS gel, and their molar concentration determined through amino acid analysis. These standard solutions were used to quantitate the amount of SR $\alpha$  and SR $\beta$  in pancreatic microsomes. Microsomal vesicles were mixed with known amounts of purified SRP receptor, and the mixture was subjected to SDS PAGE and blotted onto nitrocellulose. The blots were probed with either anti-SR $\alpha$  or anti-SR $\beta$ , followed by secondary radioactive antibodies and quantitated by counting the radioactivity in the obtained bands on the blots. Fig. 10 demonstrates that this analysis gives a linear response to added SRP receptor. Thus we could deduce the amount of SR $\alpha$  and SR $\beta$  present in the microsomal vesicles by extrapolating the curve to zero cpm. These calculations show that 1 eq (as defined in reference 28) of microsomal membranes contained 6.4 ng (93 fmol) of SR $\alpha$  and 3.2 ng (107 fmol) of SR $\beta$  or  $\sim 1.1$  mol of SR $\beta$  per mole of SR $\alpha$ . We conclude that SR $\beta$  is present in microsomal membranes equimolar to or in slight excess of SR $\alpha$ . The absolute concentration of SR $\alpha$  is in good agreement with previous rough estimates from band intensities in stained polyacrylamide gels (100 fmol per eq [12]).

If SR $\beta$  is a true subunit of the SRP receptor and as such forms a vital component of the protein translocation apparatus in the rough ER, it should also be concentrated in that particular organelle. We have therefore attempted to characterize the localization of SR $\alpha$  and SR $\beta$  in the ER membrane system. We decided to fractionate a rat liver microsomal fraction on an equilibrium sucrose gradient. Rat liver was chosen because the tissue contains substantial amounts of

both rough and smooth ER, and cell fractionation procedures are well established. Gradients were fractionated and analyzed for protein (Fig. 11 B), RNA (mostly representing membrane-bound ribosomes, Fig. 11 C), or phospholipid distribution (Fig. 11 D). Note that smooth and rough ER band as two broad, overlapping peaks that are visible in the protein and phospholipid distribution, but are not resolved.

We next determined the distribution of SR $\alpha$  and SR $\beta$  across the gradient profile using Western blot analysis. Detected bands were quantitated either by counting bound radioactive secondary antibody or by densitometric scanning of the film (see legend to Fig. 11). Upon such an analysis SR $\alpha$  (Fig. 11 E) and SR $\beta$  (Fig. 11 F) show an essentially identical distribution in the gradient fractions. To compare protein distributions between smooth and rough ER quantitatively, we define a rough ER fraction as fractions 11 and 12 (the peak of the RNA profile,  $d = 1.21$ – $1.22$  g/ml) and a smooth ER fraction as fractions 6 and 7 (the peaks of the phospholipid and protein distributions,  $d = 1.16$ – $1.17$  g/ml) (see Table I). Measurements for proteins and RNA were then normalized to the phospholipid content (which reflects the vesicle concentration and thereby the relative membrane area), and their relative distributions are given in Table I. These data show that SR $\alpha$  and SR $\beta$  are two- to threefold enriched in the rough ER fraction, however, are present in significant amounts in the smooth ER fraction as well. Unfortunately, our anti-mp30 serum does not cross-react with a corresponding rat protein. We have therefore been unable to determine a similar distribution profile for mp30.

For comparison, we analyzed the distribution for two additional proteins: ribophorin II (Fig. 11 G) (1, 21) and



**Figure 10.** Quantitation of SR $\alpha$  and SR $\beta$ . The absolute amounts of SR $\alpha$  (A) and SR $\beta$  (B) in canine microsomal membranes were determined. Increasing amounts of SRP receptor were added to 5 eq (A) or 3 eq (B) of salt-extracted microsomal membranes. The samples were subjected to SDS PAGE, blotted onto nitrocellulose, and probed with immunoselected polyclonal antibodies to SR $\alpha$  (A) and SR $\beta$  (B). Bands were quantitated by cutting from the blot and counting the radioactivity in bound secondary antibody. The amounts of the SRP receptor subunits present in the microsomal membranes were determined by extrapolating the obtained curves to zero cpm (dashed line).

NADPH cytochrome *c* reductase (Fig. 11 H) (3). Note that their distribution profiles are distinct from those of the SRP receptor subunits. Ribophorin II is enriched  $\sim 4.5$ -fold in the rough ER fraction (Table I) and its distribution appears to parallel that of RNA (i.e., the ribosome content of the vesicles) as previously reported (21). In contrast, NADPH cytochrome *c* reductase (a standard ER marker enzyme) follows the phospholipid distribution and is not enriched in the rough ER fraction, but uniformly dispersed in the ER membrane system.

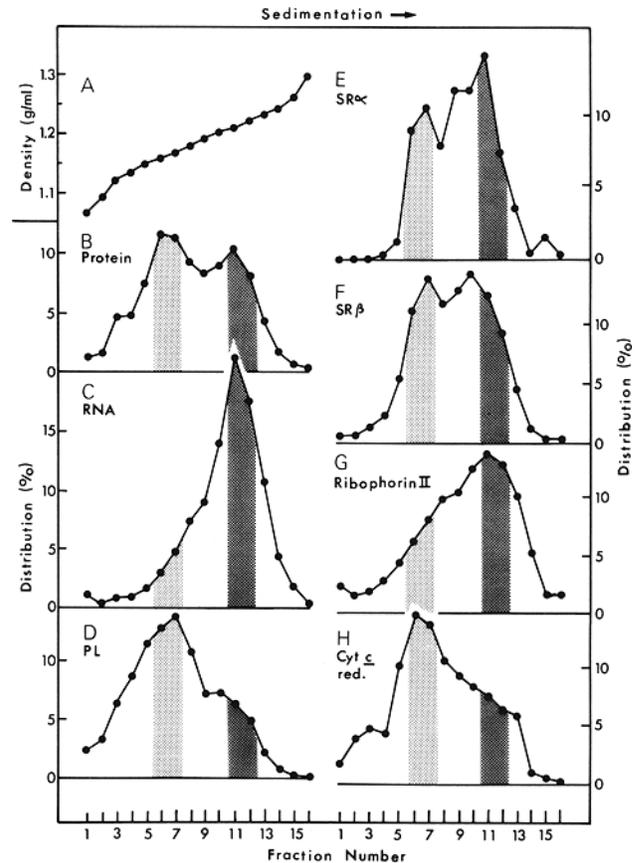
## Discussion

We report here the identification and purification of two previously undescribed ER membrane proteins of  $\sim 30$  kD. Both proteins, SR $\beta$  and mp30, have been characterized because of their affinity to known constituents of the protein translocation machinery of the ER. This makes them likely candidates to be constitutive or modulatory components of this machinery.

The first 30-kD protein, SR $\beta$ , was always found in tight association with SR $\alpha$ . Both SR $\alpha$  and SR $\beta$  are present in membranes in about equimolar amounts and co-purify by a variety of techniques described in this paper. Furthermore, the two polypeptides can be chemically cross-linked (Zimmerman, D. L., S. Tajima, and P. Walter, unpublished observations). We therefore conclude that the SRP receptor is a protein with quaternary structure, consisting of two distinct subunits. To our knowledge both subunits have been present in all previous SRP receptor preparations and have not yet been separated under non-denaturing conditions by us or others. It therefore remains to be experimentally determined whether either subunit alone can functionally interact with SRP.

If SRP receptor consists of two distinct subunits, why was only the  $\alpha$ -subunit detected previously? We attribute this oversight to the following coincidences. SRP receptor is a relatively rare protein (estimated abundance 0.1% of the ER membrane proteins), and early analysis by SDS PAGE was

performed with rather limiting amounts of material. A 30-kD "contaminant" was detected by Gilmore et al. (12), but was shown to sediment off-set from the peak of arrest releasing activity. This off-set migration on the sucrose gradient was likely due to the presence of two unresolved proteins in the same molecular weight range (SR $\beta$  and mp30), leading to a broad peak. Only the availability of specific antibodies allowed us to conclusively distinguish the two, closely spaced 30-kD proteins (Fig. 2). Additional confusion was introduced due to the presence of proteolytic breakdown products of SR $\alpha$  in the 30-kD molecular mass range, leading to the



**Figure 11.** Subfractionation of rat liver microsomes by equilibrium density centrifugation. Rat liver microsomes were fractionated as described in Materials and Methods on an equilibrium sucrose gradient. Fractions were analyzed for their density by determining the refractive index (A), for their protein (B), RNA (C), phospholipid (D), and NADPH cytochrome *c* reductase (H) content. To determine the distribution of SR $\alpha$  (E), SR $\beta$  (F), and ribophorin II (G), gradient fractions were subjected to SDS PAGE, blotted onto nitrocellulose, and probed with the respective antibodies. The data shown in F and G were determined by counting the radioactivity in bound secondary antibodies. The data in E were determined by densitometry of the autoradiogram exposed on preflashed film. This was necessary because the antibody used to detect SR $\alpha$  (shown is 12B4 [26]; a polyclonal rabbit antibody gave identical results) is only weakly cross-reactive with the rat protein. The distribution curves shown in the figure are normalized to 100%. The absolute recoveries (100%) were 16.3 mg for protein, 213  $\mu$ g for RNA, 7.5 mg for phospholipid, and 2.0 U of NADPH cytochrome *c* reductase activity. The shaded bars indicate the peak fractions of smooth and rough ER, respectively, that were used for the calculations in Table I.

**Table I. Distribution of Protein, RNA, Phospholipid, SR $\alpha$ , SR $\beta$ , Ribophorin II, and NADPH Cytochrome *c* Reductase between Rough and Smooth Microsomal Fractions.**

	Smooth ER fraction	Rough ER fraction	Rough ER fraction Smooth ER fraction
Protein	0.60	1.12	1.9
RNA	0.31	2.33	7.5
Phospholipid	1.00	1.00	1.0
SR $\alpha$	0.60	1.70	2.8
SR $\beta$	0.61	1.28	2.1
Ribophorin II	0.34	1.53	4.5
NADPH cytochrome <i>c</i> reductase	1.04	1.18	1.1

The data were taken from Fig. 11. Smooth and rough ER fraction indicate the percent recovery into fractions (6 + 7) and into fractions (11 + 12), respectively, that were normalized to the phospholipid in these fractions. The ratio of rough ER fraction to smooth ER fraction shows the relative enrichment of each component into the rough ER fraction.

report by Hortsch et al. claiming that the 30-kD band was a breakdown product of SR $\alpha$  (14). These results are reconciled with the data shown in Fig. 7 that are described in the Results section. We therefore feel that our results are not in conflict with previous reports, but rather that data that appear conflicting on first sight can be rationalized satisfactorily.

Our finding that the SRP receptor is a two subunit protein refines but does not drastically change our views on its structure or membrane disposition. In the primary sequence of SR $\alpha$  a predominantly basic domain consisting of mixed charge clusters was suggested to provide a binding site for SRP that could function through a direct interaction with 7SL RNA (20). This conjecture remains a viable hypothesis and is not affected by the presence of the additional subunit. In the primary sequence of SR $\alpha$  we also identified two potential membrane-spanning segments. Each of these regions—if they formed a membrane-spanning  $\alpha$ -helix—would place a positively charged amino acid in the middle of the hydrophobic core of the membrane. Association of SR $\alpha$  with SR $\beta$  could provide the necessary countercharges and stabilize the receptor–membrane interaction. SR $\beta$  remains intact and membrane associated after proteolytic removal of the cytoplasmic domain of SR $\alpha$  (data not shown). Therefore it is possible that SR $\beta$ , in conjunction with the remaining fragment of SR $\alpha$ , could provide the binding site to which the 52-kD cytoplasmic fragment of SR $\alpha$  can rebind noncovalently to restore a functional receptor.

The second newly identified 30-kD membrane protein, mp30, was purified on SRP-Sepharose and binds to SRP directly. This interaction also appears to be specific. On the SRP-Sepharose column, binding of mp30 saturated at the same molar stoichiometry as the SRP–SRP receptor interaction. SRP receptor and mp30 must however bind to different sites on SRP since they do not compete with each another for binding (Fig. 1). Furthermore, mp30 was eluted from SRP-Sepharose under the same conditions used to elute SRP receptor by increasing the magnesium concentration in the buffer from 5 to 25 mM, while the monovalent cation concentration was adjusted to keep the ionic strength constant. These conditions, originally described by Gilmore et al. (11), appear to be subtle, possibly affecting conformational changes in SRP, and are unlikely to cause elution if binding were due to nonspecific ionic interactions. Nevertheless, we have presently no means to distinguish whether the binding affinity reflects a physiologically meaningful interaction or is merely fortuitous. Purified mp30 in detergent solution had

no elongation arrest releasing activity when assayed in vitro, nor did it measurably promote or inhibit the activity of SRP receptor (Lauffer, L., unpublished observations). Antibodies to mp30 did not inhibit the protein translocation activity of microsomal membranes (Tajima, S., unpublished observations). We do not know, however, whether our antibodies are directed towards cytoplasmically exposed epitopes on mp30. Thus, while mp30 remains a good candidate for a functionally important protein, functional assays will be required to assess its putative role in protein translocation. One can speculate that additional SRP-binding proteins in the ER membrane could act, for example, to locally increase the SRP concentration. Alternatively they may directly participate in the SRP targeting cycle or be involved in recycling SRP after it has interacted with its receptor.

SRP receptor was previously claimed to be a marker protein specific for the rough membranes of the ER (13, 15). Smooth and rough ER form a continuous membrane system and are morphologically and experimentally distinguished by their density of membrane-bound ribosomes. Most if not all of these ribosomes are actively translating proteins, and translation appears to be coupled to translocation of the nascent polypeptides across the ER membrane. Upon separation of smooth and rough ER by equilibrium sucrose gradient centrifugation it was unexpected, in contrast to the above-mentioned claim, to find that SRP receptor was present at relatively high concentrations in the light ER fractions. Both ribosomes (measured as RNA) and ribophorin II (a protein that was suggested to function as a ribosome receptor [32]) show a much more skewed distribution toward the heavier gradient fractions than does SRP receptor. This implies that a population of SRP receptor (amounting to about half the SRP receptor molecules in a rat liver cell) exists in regions of the ER with a low ribosome density and a low ribophorin concentration. These sites may represent regions of the ER to which newly initiated polysomes are targeted. Ribophorins and possibly other ribosome-binding proteins may act subsequently to stabilize those ribosomes that are already functionally engaged on the membrane to eventually establish a classical ribosome–membrane junction.

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