The Signal Sequence Receptor, Unlike the Signal Recognition Particle Receptor, Is Not Essential for Protein Translocation

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Abstract. Detergent extracts of canine pancreas rough microsomal membranes were depleted of either the signal recognition particle receptor (SR), which mediates the signal recognition particle (SRP)-dependent targeting of the ribosome/nascent chain complex to the membrane, or the signal sequence receptor (SSR), which has been proposed to function as a membrane bound receptor for the newly targeted nascent chain and/or as a component of a multi-protein translocation complex responsible for transfer of the nascent chain across the membrane. Depletion of the two components was performed by chromatography of detergent extracts on immunoaffinity supports. Detergent extracts lacking either SR or SSR were reconstituted and assayed for activity with respect to SR dependent elongation arrest release, nascent chain targeting, ribosome binding, secretory precursor translocation, and membrane protein integration. Depletion of SR resulted in the loss of elongation arrest release activity, nascent chain targeting, secretory protein translocation, and membrane protein integration, although ribosome binding was unaffected. Full activity was restored by addition of immunoaffinity purified SR before reconstitution of the detergent extract. Surprisingly, depletion of SSR was without effect on any of the assayed activities, indicating that SSR is either not required for translocation or is one of a family of functionally redundant components.

P ROTEIN translocation across the RER occurs through a series of discrete stages. Identified reaction steps include: (*a*) the signal sequence-dependent and signal recognition particle- (SRP)[1] mediated targeting of the nascent chain to the RER membrane; (*b*) binding of the precursor to components of the RER membrane; (*c*) translocation, and in most instances; (*d*) signal sequence cleavage. Each reaction step is presumably mediated by resident integral membrane proteins. Targeting, for example, is dependent upon the SRP receptor (SR) (Gilmore et al., 1982*a,b*; Meyer et al., 1982), whereas signal sequence cleavage is catalyzed by the signal peptidase complex (Evans et al., 1986). Although translocation per se requires the activity of resident integral membrane proteins, the precise biochemical function of such proteins has yet to be defined (Hortsch et al., 1986; Nicchitta and Blobel, 1989; Hartmann et al., 1989; Yu et al., 1990; Klappa et al., 1991). It is evident, however, that protein translocation occurs through a protein conducting channel and it is thus expected that specific membrane

components would function either as subunits of such a channel or in the regulation of channel activity (Blobel and Dobberstein, 1975; Singer et al., 1987; Simon and Blobel, 1991).

Recently, procedures were developed for the complete solubilization of RER vesicles and subsequent reconstitution of translocation activity (Nicchitta and Blobel, 1990; Nicchitta et al., 1991*a*). By providing biochemical accessibility to both the protein and lipid components of the RER membrane, the detergent solubilized system can be used in the identification of the biochemical activities responsible for translocation. The described reconstitution procedures can also be exploited to define the functional contribution of previously identified RER-specific membrane components to protein translocation.

In this report we describe the results of studies on the depletion and functional analysis of two membrane protein complexes, SR, and the signal sequence receptor (SSR). There is substantial experimental evidence in support of an essential function for both SR and SSR in protein translocation. SR was initially identified as a membrane-bound receptor for SRP and it has been clearly demonstrated that the cytoplasmic domain of the α subunit is required for release of SRP from the ribosome/nascent chain complex, a prerequisite for translocation (Gilmore et al., 1982*a,b*; Gilmore and Blobel, 1983; Connolly and Gilmore, 1989). SSR was iden-

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1. *Abbreviations used in this paper:* CE, cholate extract; DSP, dithiobis (succinimidyl propionate); DSS, disuccinimidyl suberate; EA, acid eluate; EP, peptide eluate; FT, flow through; GLO, globulin; MBS, maleimido bis-succinimide ester; PL, prolactin; pPL, preprolactin; RM, rough microsomes; SR, signal recognition; SRP, signal recognition particle; SSR, signal sequence receptor; sulfo-MBS, sulfosuccinimidoester.

tified in a series of cross-linking experiments as a protein residing in the immediate vicinity of the signal sequence of the nascent chain during the initial, SRP-dependent targeting event (Wiedmann et al., 1987). Subsequent studies have also indicated that SSR is in close physical proximity to both nascent secretory as well as membrane protein precursors during translocation (Krieg et al., 1989; Wiedmann et al., 1989; High et al., 1991; Thrift et al., 1991). It has also been demonstrated that either IgG or Fab fragments directed against the cytoplasmically exposed carboxy terminus of the α subunit block translocation of the secretory precursor preprolactin (Hartmann et al., 1989).

We have observed that whereas SR is essential for the initial targeting and binding of the nascent chain, and thus for translocation itself, depletion of SSR is without effect on either elongation arrest release, nascent chain targeting, ribosome binding, protein translocation, or membrane protein integration.

Materials and Methods

Preparation of Rough Microsomes, SRP, and Detergent Extracts

Rough microsomes (RM) were prepared from canine pancreas by the procedure of Walter and Blobel (1983a). The RM suspension, at 50 A_{280} U/ml, was sequentially washed in a low salt buffer and in a high salt–EDTA buffer as described previously (Nicchitta and Blobel, 1990). The washed membranes (RMek) were resuspended in 0.25 M sucrose, 50 mM triethanolamine, pH 7.5 (TEA), 1 mM DTT, and stored at −80°C.

SRP was prepared as described in Walter and Blobel (1983b).

Detergent extracts were prepared as described in Nicchitta and Blobel (1990) with the following modifications: a 10-ml aliquot of RMek was overlaid on a 12-ml sucrose cushion (0.5 M sucrose, 50 mM TEA, 1 mM DTT) and centrifuged in a Beckman TI 50.2 rotor for 60 min at 150,000 g_{av} (4°C). The membranes were resuspended in 9 ml of solubilization buffer (0.4 M sucrose, 0.45 M KCl, 20 mM Tris-Cl, pH 7.6, 1 mM MgCl$_2$, 0.4 mM DTT, 0.1 mM MgATP, 0.1 mM MgGTP, 20 μg/ml antipain, 20 μg/ml chymostatin, 5 μg/ml leupeptin, 5 μg/ml pepstatin, and 66 KIU/ml trasylol), gently homogenzied with a glass teflon homogenizer, and solubilized by the addition of sodium cholate to a concentration of 0.75% (wt/vol). The detergent-treated membranes were incubated on ice for 30 min, and a high speed supernatant (CE) was prepared by centrifugation for 70 min at 225,000 g_{av} (4°C) in a rotor (model T875; Sorvall Instruments, Newton, CT). Following centrifugation, the CE was removed and stored on ice.

Peptides and Antibodies

The peptides used for this study were synthesized and partially purified (80–90%) by the Rockefeller University protein sequencing facility or by Multiple Peptide System (La Jolla, CA). All peptides were synthesized with a cysteine residue at either the NH$_2$ or the COOH terminus for subsequent chemical coupling.

Peptide SRα. Representing amino acids 137–150 of the SRP receptor α subunit (Lauffer et al., 1985).

Peptide SSRα. Representing the COOH-terminal 15 amino acid of the α subunit of the signal sequence receptor (Prehn et al., 1990).

Peptide SSRγ. Representing amino acids 1–14 (NH2-A-P-K-G-G-S-K-Q-Q-S-E-E-D-L-C-COOH) of the γ subunit of the signal sequence receptor, obtained by automated sequencing of the purified protein (G. Migliaccio, C. Nicchitta, and G. Blobel, unpublished results).

Peptide SSRδ. Representing amino acid 5–18 (NH2-E-P-Q-I-T-P-S-Y-Y-T-T-S-D-A-C-COOH) of the δ subunit of the signal sequence receptor, obtained by automated sequencing of the purified protein (G. Migliaccio, C. Nicchitta, and G. Blobel, unpublished results).

Peptides were conjugated to keyhole limpet hemocyanin with maleimido bis-succinimide ester (MBS; Pierce Chemical Co., Rockford, IL) and used for the preparation of rabbit polyclonal antisera as described in Harlow and Lane (1988). mAbs to the α and β subunit of SR (Tajima et al., 1986) were

a kind gift of Dr. Peter Walter (University of California, San Francisco, CA). The rabbit polyclonal antisera to the 22/23-kD subunit of the canine signal peptidase complex (Shelness et al., 1986) and the α subunit of SSR (Wada et al., 1991) were a kind gift of Dr. Greg Shelness (Wake Forest University, Winston-Salem, NC) and Dr. John Bergeron (McGill University, Montreal Canada), respectively. The rabbit polyclonal antisera to the β subunit of SSR was raised against the SDS-denatured protein, purified as described in Hartmann et al. (1989).

Affinity Purification of Antibodies and Crosslinking to Protein-A Sepharose

The described peptides were coupled to ω-aminohexyl-agarose (Sigma Chemicals Co., St. Louis, MO) with MBS at a density of 10 mg of peptide per ml of agarose and used to affinity purify the respective antibodies as described in Harlow and Lane (1988). Affinity purified IgGs to either SRα or SSRα peptides were crosslinked to protein A-Sepharose with dimethyl pimelimidate at a ratio of 8 mg of IgG per ml of resin. Coupling conditions were as described in Harlow and Lane (1988).

Immunodepletion of Cholate Extract

1.0-ml IgG-protein-A Sepharose columns were extensively washed with acid buffer (0.1 M glycine/HCl, pH 3.0, 1% CHAPS) and equilibrated with 10 vol of column buffer (solubilization buffer containing 0.75% sodium cholate and 0.8 mg/ml phosphatidylcholine). 8 ml of cholate extract (CE) were loaded on the columns at a flow rate of 1 ml/h. The flow through (FT) was collected in 1 ml fractions and frozen in liquid nitrogen. The columns were washed with 30 ml of column buffer at a flow rate of 15 ml/h and eluted with 8 ml of column buffer containing 100 μg/ml of the appropriate peptide at 1 ml/h. The peptide eluate (EP) was collected in 0.5-ml fractions and frozen in liquid nitrogen. Subsequently, the columns were washed with solubilization buffer containing 1% CHAPS and eluted with 5 ml of acid buffer at a flow rate of 2.5 ml/h. CHAPS, a zwitterionic relative of cholate, was substituted for cholate in all conditions requiring acidic pH to avoid detergent precipitation. The acid eluate (EA) was collected in 1-ml fractions and frozen in liquid nitrogen after adjustment of the pH to 7.0.

Reconstitution of Vesicles

Reconstitution of protein subfractions into proteoliposomes was accomplished by the detergent extraction procedure (Nicchitta et al., 1991b). For this procedure, SM 2 BioBeads (Bio-Rad Laboratories, Richmond, CA) were sequentially washed in methanol, water, and solubilization buffer. Approximately 1.2 ml of washed Bio-Beads were packed in disposable polypropylene columns and, immediately before reconstitution, centrifuged to remove excess buffer. Samples for reconstitution were prepared as follows. 400-μl aliquots of either CE, SRα-depleted flow through or SSRα-depleted flow through were supplemented with 200 μl of column buffer or, where indicated, with 200 μl of peptide specific eluate from the anti-SRα column (containing ∼2 eq of SRα/μl). Samples were incubated on ice for 30 min and subsequently loaded onto the Bio-Bead columns. Samples were incubated for 1 h on ice, with occasional mixing, to allow binding of the detergent by the hydrophobic resin. Reconstituted vesicles were recovered by centrifugation of the columns for 2 min at 1,000 g, the eluate diluted with 1 vol of 0.6 M KCl, 50 mM TEA, 1 mM DTT, and collected by centrifugation for 20 min at 110,000 g_{av} in a rotor (TL45; Beckman Instruments, Inc., Palo Alto, CA). Membranes were resuspended in 200 μl of 0.25 M sucrose, 50 mM TEA, 1 mM DTT, homogenized with a teflon pestle, frozen in liquid nitrogen, and stored at −80°C.

In Vitro Transcription and Translation

mRNAs coding for full length or truncated (86-mer) bovine preprolactin were transcribed with T7 RNA polymerase from plasmid pGEMBP1 (Connolly and Gilmore, 1986). mRNA coding for full-length Sindbis E1 protein was transcribed with SP6 RNA polymerase from plasmid pSP1900 (Migliaccio et al., 1989). In vitro translation reactions (25 μl) were performed for 45 min at 25°C using either a nuclease-treated reticulocyte lysate or a wheat germ translation system. mRNAs coding for full length preprolactin and Sindbis E1 protein were used at 100 ng per reaction; mRNA coding for preprolactin 86 mer was used at 300 ng per reaction. Translation conditions were as described (Nicchitta and Blobel, 1989). After translation, samples were chilled on ice and proteins were precipitated either with 10% TCA (wheat germ translations) or with 66% ammonium sulfate

(reticulocyte lysate translations), and then prepared for SDS-PAGE as described (Nicchitta and Blobel, 1989).

Elongation Arrest-releasing Activity

Elongation arrest-releasing activity was assayed as described by Gilmore et al. (1982a) with minor modifications. Preprolactin mRNA (100 ng per reaction) and rabbit reticulocyte α and β globin mRNAs (50 ng per reaction) were translated in a cell free wheat germ system in the presence or absence of 16 nmolar SRP. The translations were further supplemented with reconstituted membranes at a concentration of 4 or 8 nmoles of phospholipid per 25-μl reaction. After translation, the reactions were chilled on ice, precipitated with 10% TCA, resuspended in sample buffer and prepared for SDS-PAGE as described (Nicchitta and Blobel, 1990). Incorporation of radioactivity into specific precursors was determined by direct analysis of the dried gel with an AMBIS Radioanalytic Imaging System (Automated Microbiology Systems, Inc., San Diego, CA). Incorporation of [^{35}S]methionine into prolactin was corrected for the loss of one methionine residue following cleavage of the signal peptide. The incorporation of [^{35}S]methionine into preprolactin (pPL) and prolactin (PL) was normalized with respect to the incorporation of [^{35}S]methionine into globin (GLO) which served as an internal standard in the elongation arrest release assay. The percent inhibition by SRP of pPL synthesis was calculated as follows:

$$\% \text{ inhibition } = [1-(R^+/R^-)] \times 100$$

where:
$R^- = (\text{pPL} + 8/7 \text{ PL})/\text{GLO}$ in the absence of SRP
$R^+ = (\text{pPL} + 8/7 \text{ PL})/\text{GLO}$ in the presence of SRP

and pPL, PL, and GLO are the cpm incorporated into preprolactin, prolactin, and globin, respectively. The percent processing of preprolactin was calculated with the following formula:

$$\% \text{ processing } = 8/7 \text{ PL}/(\text{pPL} + 8/7 \text{ PL}).$$

Ribosome Binding

Ribosome binding studies were performed as described in Nicchitta et al. (1991) using [^{3}H]uridine-labeled ribosome prepared from HeLa cells as described in Kreibich et al. (1983). Aliquots of reconstituted vesicles containing 20 μg of protein were incubated for 1 h on ice with 20 μg of [^{3}H]uridine-labeled ribosomes (1.4 $\times$ 10^4 cpm/μg protein) in 50 μl of 50 mM Tris-Cl, pH 7.5, 25 mM KCl, 5 mM MgCl$_2$ (buffer A). Subsequently, 450 μl of 2.3 M sucrose in buffer A was added and the samples were layered under a discontinuous sucrose gradient consisting of 1 ml of 1.9 M sucrose, 1 ml of 1.6 M sucrose, 0.75 ml of 1.3 M sucrose, all prepared in buffer A, and 1.25 ml of buffer A. Gradients were centrifuged for 3 h at 287,000 g_{av} in a rotor (SW55; Beckman Instruments, Inc., Fullerton, CA). Gradients were fractionated into 0.5-ml fractions and a pellet fraction, with a Buchler Auto-Densi flow gradient harvester (Buchler Instruments, Lexana, KN). After dilutions to 1 ml with 2 mg/ml BSA, samples were precipitated with 10% TCA and collected by vacuum filtration onto Whatman GF/C glass-fiber filters (Whatman International, Ltd., Maidstone, England). Radioactivity was determined by liquid scintillation counting of the filters in Beckman Ready-Safe scintillation cocktail (Beckman Instruments, Inc.).

Nascent Chain Targeting

Wheat germ translation reactions, using the truncated (86 mer) pPL mRNA, were performed in the presence of reconstituted vesicles at a concentration of 4 nmoles of phospholipid per reaction. After translation, reactions were chilled on ice and supplemented with 175 μl of 28.5 mM EDTA, 120 mM KoAC, and 50 mM TEA. After an additional 10-min incubation on ice, samples were loaded onto a 200-μl cushion (0.5 M sucrose, 25 mM EDTA, 120 mM KOAc, 50 mM TEA) and centrifuged for 10 min at 175,000 g_{av} in a rotor (TL100; Beckman Instruments, Inc.). The supernatants, including the cushion, were removed and precipitated with 10% TCA. Both pellet and supernatant fractions were then resuspended directly in sample buffer and analyzed by SDS-PAGE and autoradiography.

Protease Protection Assay

Translation–translocation assays were chilled on ice and supplemented with 3 mM tetracaine (Scheele, 1983). Proteinase K was added to a final concentration of 100 μg/ml and digestions were performed for 30 min on ice. After protease digestion, PMSF was added to a final concentration of 10 mM and,

after an addition 10 min, proteins were precipitated with ammonium sulfate and prepared for SDS-PAGE as described previously (Nicchitta and Blobel, 1989).

Membrane Protein Integration

Reticulocyte lysate translation reactions (25 μl), programmed with Sindbis E1 mRNA, were performed in the presence of RMek or reconstituted vesicles at a concentration of 4 nmoles phospholipid per reaction. After translation, reactions were chilled on ice and supplemented with 20 vol of ice cold 5.25 M Urea, 50 mM TEA pH 7.5. After a 10-min incubation on ice, 200 μl of each sample was loaded onto a 200-μl cushion (0.25 M sucrose, 4.5 M Urea, 50 mM TEA, pH 7.5) and centrifuged for 15 min at 225,000 g_{av} in a rotor (TL100; Beckman Instruments, Inc.). The supernatants, including the cushion, were removed and precipitated with ammonium sulfate as described above. Pellets and precipitates were washed with 5% TCA resuspended in sample buffer and prepared for SDS-PAGE as described above.

General Procedures

Protein concentrations were determined by the bicinchoninic acid assay using BSA as a standard (Pierce Chemical Co., Rockford, IL). Phospholipid concentrations were determined by the procedure of Ames and Dubin (1960) with inorganic phosphate as a standard.

Materials

[^{35}S]Methionine and [^{125}I]protein A were from New England Nuclear (Boston, MA). T7 and SP6 RNA polymerases, nuclease-treated rabbit reticulocyte lysate, wheat germ lysate, and RNasin, a placental RNase inhibitor, were obtained from Promega Biotec (Madison, WI). ATP, GTP, and 1-deoxynojirimycin, a glucosidase I and II inhibitor were from Boehringer-Mannheim Biochemicals (Indianapolis, IN). Protein A Sepharose fast flow was from Pharmacia (Uppsala, Sweden). Na-cholate, Ultrol grade, was from Calbiochem-Behring Corp. (La Jolla, CA). α and β globin mRNA was obtained from Bethesda Research Laboratories (Bethesda, MD). Glycosylation acceptor peptide was the generous gift of Dr. Martin Wiedmann (Sloan-Kettering Cancer Center, New York, NY). All other reagents were of the highest quality commercially available.

Results

Immuno-depleted Detergent Extracts

Detergent extracts depleted of either the SR or the SSR were prepared by chromatography on antibody supports prepared from affinity purified anti–peptide IgG fractions. Column fractions corresponding to the CE, the column FT, the EP, and the EA were separated by SDS-PAGE, transferred to nitrocellulose, and the blots either stained with amido-black (Fig. 1 A), or analyzed with antisera (Fig. 1 B) directed against either the 22/23-kD glycoprotein subunit of the signal peptidase complex (SP 22/23), the α and β subunits of SR, or the α, β, γ, and δ subunits of SSR. Note that with respect to SSRα, immunoblots were performed with antisera directed against a synthetic peptide representing the carboxy-terminal 15 amino acids (SSRα[pept]) as well as with antisera prepared against the SDS-denatured protein (SSRα[prot]).

Chromatography of the CE on the immunoaffinity supports resulted in specific depletion of either SR or SSR. As shown in Fig. 1 A, lane 3, elution from the anti-SRα column with the appropriate peptide yielded a fraction highly enriched in the α (72 kD) and β (30 kD) subunits of SR. Proteins of 34, 25, 21, and 19 kD are observed upon elution with the appropriate peptide from the anti-SSRα column (Fig. 1 A, lane 6). Amino-terminal sequence analysis of the 21- and 19-kD proteins (see Materials and Methods) reveals no homology with previously described proteins (G. Migliaccio,
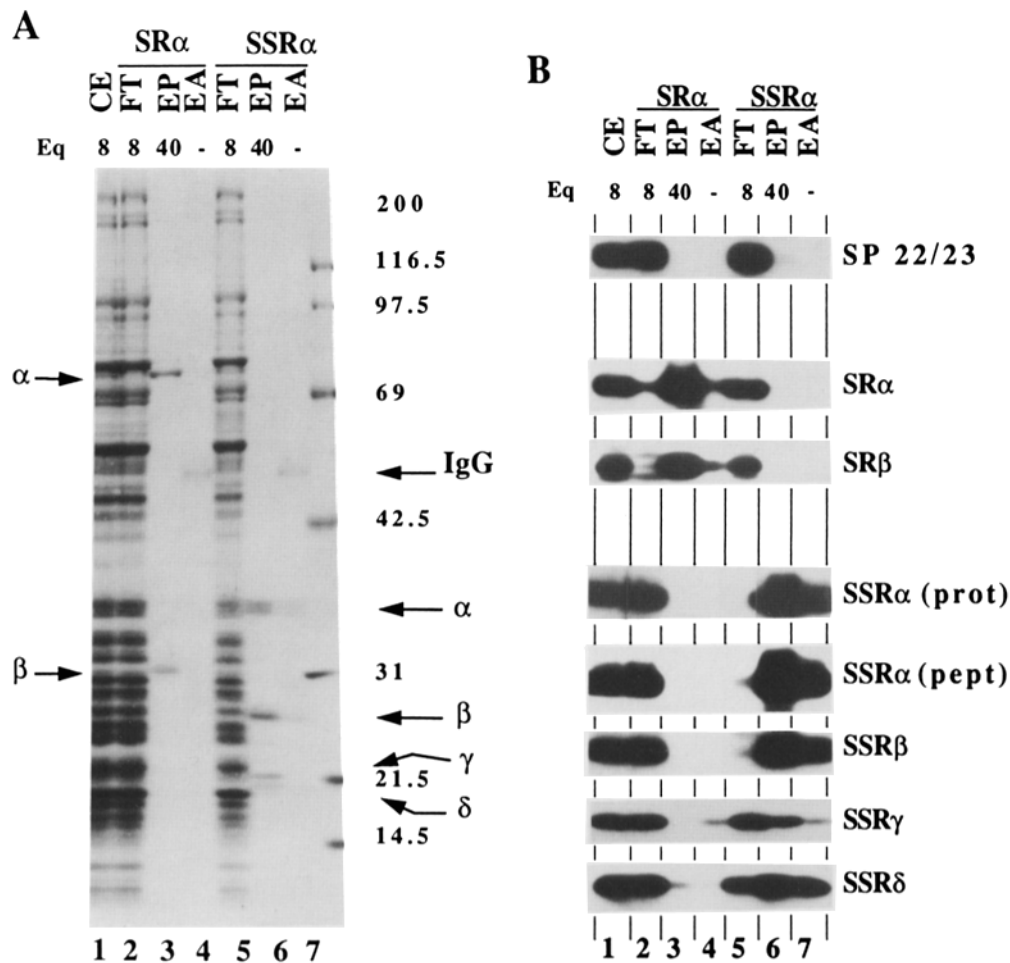
Figure 1. Immunodepletion of SRP receptor SR and SSR from solubilized rough microsomal membranes. Salt–EDTA-washed rough microsomes were solubilized with sodium cholate and a high speed supernatant, referred to as CE was prepared. Aliquots of the CE were fractionated on 1-ml affinity column containing either IgG directed against the α subunit SR or the α subunit of SSR. Proteins from the FT fraction and those eluted by the corresponding peptide (*EP*) or eluted by acid (*EA*), were separated by 8–13% gradient SDS-PAGE and electrophoretically transferred to nitrocellulose. (*A*) The blots were stained with amidoblack. The positions of the SR and SSR subunits are indicated to the left and right, respectively. IgG denotes the position of IgG heavy chain. Relative molecular weight standards are shown in the rightmost lane. (*B*) Blots were probed with antibodies directed against the 22/23-kD subunit of the signal peptidase complex (*SP 22/23*); the α and β subunits of SR (*SRα* and *SRβ*, respectively), the SSRα peptide (SSRα pept.); the α and β subunits of the SSR (*SSRα prot* and *SSRβ*, respectively) and the SSRγ and SSRδ peptides. Bound antibodies were detected with [^{125}I]protein A and subsequent autoradiography.

C. Nicchitta, and G. Blobel, unpublished observations). As it is likely that these two proteins correspond to proteins of similar relative molecular weight previously identified in near neighbor cross-linking studies of SSR, they are referred to as the γ (21 kD) and δ (19 kD) subunits of SSR (Görlich et al., 1990). Subsequent elution of the columns with mild acid (lanes *4* and *7*), demonstrates that the depletion was specific. The only proteins observed to elute with mild acid were IgG or SRα/β and SSRα/β/γ/δ.

The corresponding immunoblots of the column fractions show that, on the anti-SRα column, there was depletion, from the CE, of ~85% of both SRα and β, with little or no depletion of either the 22/23-kD glycoprotein component of the signal peptidase complex (SP22/23) or the α, β, γ, and δ subunits of SSR (Fig. 1 *B*, lanes *1* and *2*). As expected, elution with peptide (lane *3*) yielded both SRα and β. Detectable amounts of the two subunits were also recovered in the acid eluate (lane *4*). Chromatography of a CE on the anti-

SSRα column resulted in >95% depletion of SSRα and β with little or no depletion of SRα/β, or SP 22/23 (Fig. 1 *B*, lanes *1* and *5*). Depletion of the γ and δ subunits of SSR was relatively inefficient (lane *5*), indicating that the association of the two subunits with the α and β subunits is relatively weak under the described solubilization conditions. SSRα/β was recovered in the peptide eluate as was a significant quantity of the γ and δ subunits (lane *6*). Peptide elution of SSR from the immunoaffinity support was also somewhat inefficient, with a substantial quantity of the α, β and δ subunits and a lesser amount of the γ subunit being recovered in the acid eluate (lane *7*).

Reconstitution of Depleted Extracts: Protein Composition

Unfractionated CE and FT fractions described above were reconstituted by a detergent binding procedure to yield CE
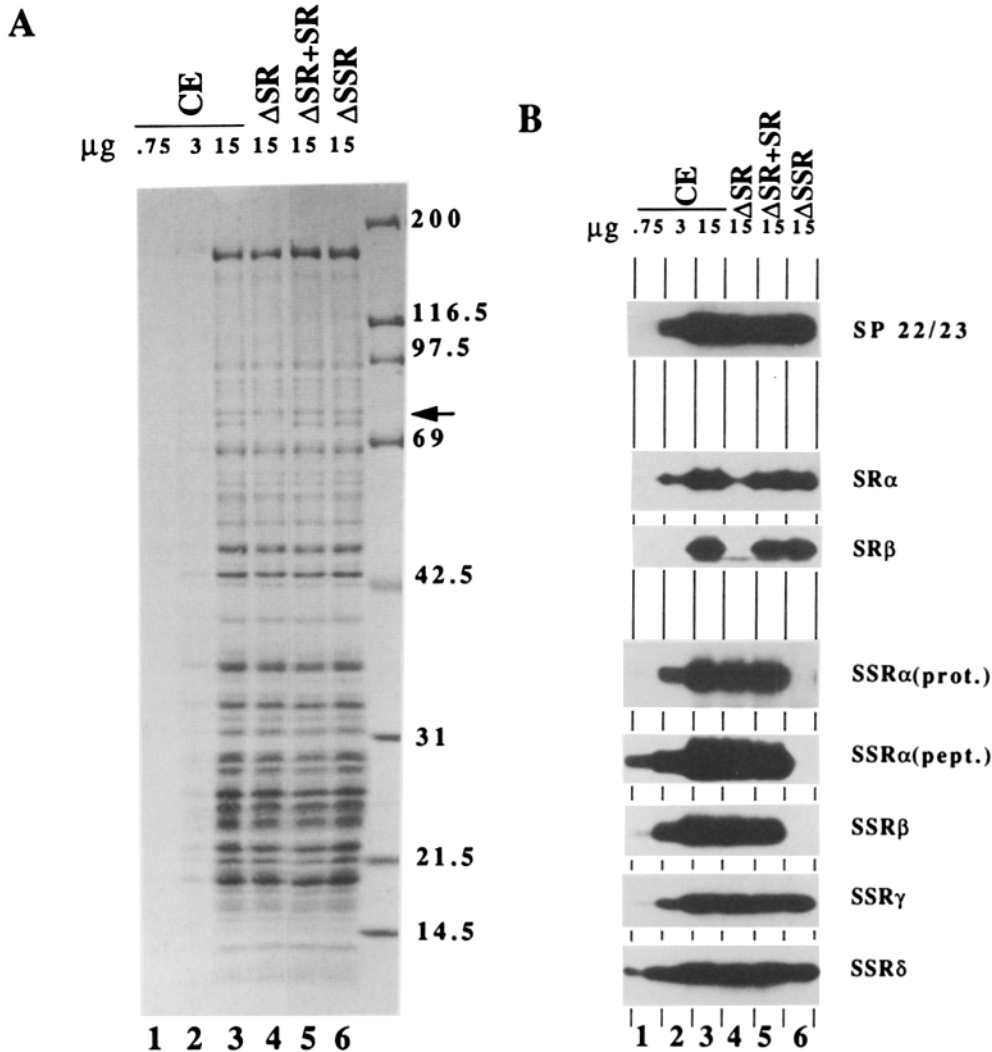
18

Figure 2. Protein composition of reconstituted vesicles. Samples generated in the immuno-depletion experiment shown in Fig. 1 were reconstituted into membranes by the detergent extraction procedure described in Materials and Methods. CE vesicles were reconstituted from CE. ΔSR vesicles were reconstituted from the FT of the SRα column. ΔSR + SR vesicles were derived from the same fraction supplemented with the EP fraction from the SRα column. ΔSSR vesicles were derived from the FT fraction of the SSRα column. Aliquots of these vesicles, containing the indicated amount of total proteins (µg), were separated by 8–13% gradient SDS-PAGE and electrophoretically transferred to nitrocellulose. The blots were either stained with amido-black (A) or probed with antibodies (B) as described in the legend to Fig. 1.

vesicles and vesicles deficient in either SRα/β (ΔSR) or SSRα/β (ΔSSR). Additionally, control vesicles were also reconstituted from an SRα/β depleted CE supplemented with the immunoaffinity purified SRα/β fraction (ΔSR + SR). The total protein composition of the various vesicle fractions (Fig. 2 A) is quite similar, with the exception of the ΔSR vesicles, of which the loss of the α subunit can be seen by amido black staining (Fig. 2 A, lane 4, arrow). ΔSR vesicles contain ~15% of the SRα present in CE (Fig. 2 B, lanes 1–3), ΔSR + SR (lane 5), or ΔSSR vesicles (lane 6). ΔSSR vesicles contain <5% of the SSRα/β observed in CE (lanes 1–3), ΔSR (lane 4), or ΔSR + SR (lane 5) vesicles. It is difficult to estimate the vesicle content of SSRγ and δ in the ΔSSR vesicles as the autoradiograms of the immunoblots were overexposed to allow more precise estimates of the efficiency of depletion of SSRα and β.

Analysis of Ribosome Binding and Elongation Arrest Release Activity

Targeting of the ribosome/nascent chain/SRP complex to the RER membrane is comprised of at least two steps: the SR mediated release of SRP from the ribosome/nascent chain

complex, and binding of the ribosome to the RER membrane (Gilmore et al., 1982a,b; Kreibich et al., 1978a; Yoshida et al., 1987; Savitz and Meyer, 1990; Nunnari et al., 1991; Collins and Gilmore, 1991). The ribosome binding activity of CE, ΔSR, ΔSR + SR, and ΔSSR vesicles is shown in Fig. 3 A. In this experiment ribosomes were present in stoichiometric excess to membrane-associated binding sites. Comparison of the ribosome binding activity observed in the presence of 20 µg of vesicle protein clearly indicates that depletion of either SR or SSR was without effect on ribosome binding.

The elongation arrest release activity of the different vesicle preparations is depicted in Fig. 3 B. Under the described assay conditions, the addition of SRP to a preprolactin programmed wheat germ translation system inhibits preprolactin synthesis by ~80%. In the presence of CE vesicles, at either 4 or 8 nmol of lipid phosphorous per reaction, a 49 and 59% release, respectively, of elongation arrest is observed. Depletion of SR yielded a substantial loss of the elongation arrest release activity, a defect that was reversed when reconstitution of the depleted extracts was performed in the presence of the purified SR (Fig. 3 B). Somewhat sur-
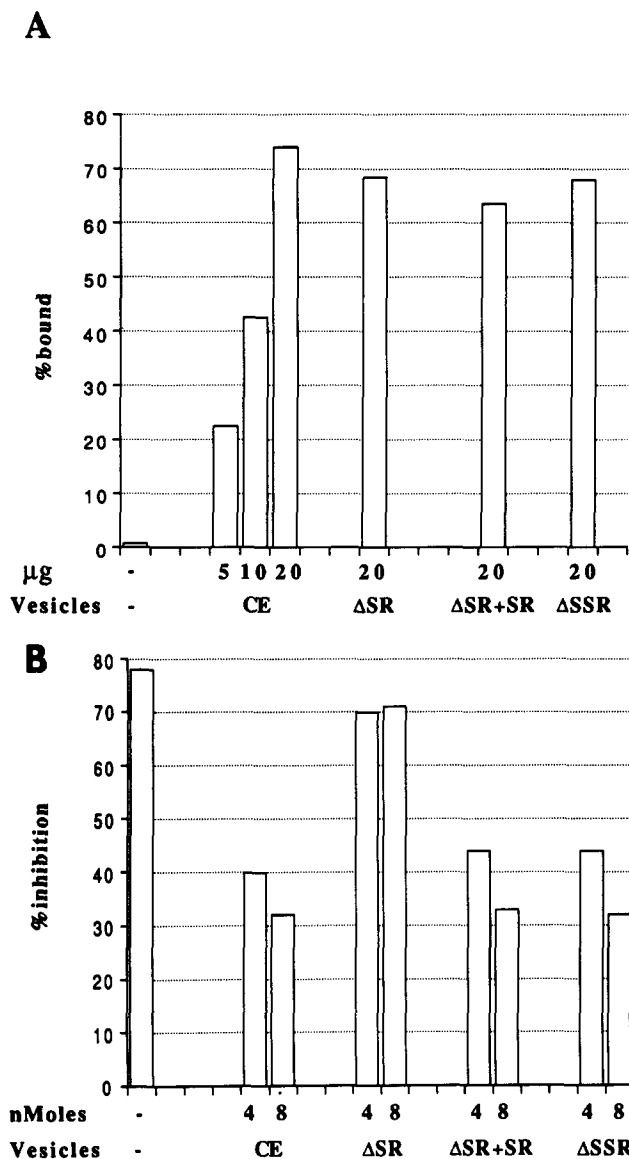
A

μg	-	5 10 20	20	20	20
Vesicles	-	CE	ΔSR	ΔSR+SR	ΔSSR

B

nMoles	-	4 8	4 8	4 8	4 8
Vesicles	-	CE	ΔSR	ΔSR+SR	ΔSSR

Figure 3. Ribosome binding and elongation arrest release activity of reconstituted vesicles. Reconstituted vesicles, defined in the legend to Fig. 2, were assayed for their ability to bind radiolabeled ribosomes (*A*) and to release the SRP-induced arrest of preprolactin synthesis (*B*). (*A*) Aliquots (5, 10, or 20 μg protein) of the indicated reconstituted vesicles were incubated with 20 μg (protein) of [³H]uridine-labeled HeLa cell ribosomes in a total volume of 50 μl for 1 h on ice. Samples were analyzed on discontinuous sucrose gradients as described in the Materials and Methods. Values are expressed as membrane bound ribosomes relative to the total recovered from the gradient. (*B*) A mixture of bovine preprolactin and rabbit α and β globin mRNA were translated in a 25 μl wheat germ system in the presence or absence of 16 nM SRP. Where indicated, translations were supplemented with the various reconstituted vesicles at a concentration of either 4 or 8 nmoles of phospholipids per reaction. Translation products were analyzed by 12.5% SDS-PAGE. The percent inhibition of prolactin synthesis was calculated as described in Materials and Methods.

prisingly, ΔSSR vesicles displayed elongation arrest release activity that was nearly indistinguishable from either CE or ΔSR + SR vesicles. It has previously been demonstrated that treatment of membranes with either IgG or Fab frag-

ments directed against the cytoplasmic COOH-terminal domain of SSRα results in a marked inhibition of both translocation and elongation arrest release (Hartmann et al., 1989). SSR was suggested to function, at least in part, as a signal sequence receptor (Hartmann et al., 1989). The antibody-dependent block of elongation arrest release was thus thought to reflect a kinetic blockade which would be predicted to appear following inhibition of signal sequence receptor activity. As indicated in Fig. 3 *B*, however, ΔSSR vesicles display little or no loss of elongation arrest release activity.

Precursor Targeting: Membrane Association

Binding of the nascent chain to the RER membrane can occur through association of the translating ribosome with the membrane and/or by a direct binding of the nascent chain to additional, presumably proteinaceous, components of the RER membrane (Gilmore and Blobel, 1985). The direct-binding reaction is GTP dependent and can readily be distinguished from ribosome-mediated binding by extraction of the membranes with EDTA, which promotes ribosome disassembly and subsequent release of the ribosome associated nascent chains (Connolly and Gilmore, 1986). To assess the functional consequences of depletion of either SR or SSR on the binding of the nascent chain, a truncated preprolactin precursor was synthesized in a wheat germ translation system in the presence or absence of SRP, and EDTA insensitive association of the nascent chain with the ΔSR, ΔSR + SR, or ΔSSR vesicles subsequently determined by sedimentation analysis. The results of these experiments are shown in Fig. 4, *A* and *B*. When the truncated preprolactin precursor (86-mer) was synthesized in the absence of SRP the nascent chain was recovered in the supernatant (*S*) fraction in either the absence of vesicles or in the presence of ΔSR, ΔSR + SR, or ΔSSR vesicles (*A*). In contrast, when synthesis was performed in the presence of SRP, a marked increase in the association of the 86-mer with the ΔSR + SR and the ΔSSR vesicles was observed (Fig. 4, compare lanes *5* and *13* and lanes *7* and *15*). As expected, ΔSR vesicles did not exhibit enhanced binding of the 86 mer in the presence of SRP (Fig. 4, compare lanes *3* and *11*). The activity of ΔSSR and ΔSR + SR vesicles was quantitatively similar to that observed for CE vesicles (data not shown). These data indicate, in agreement with previous studies, that SR is required for the SRP-dependent binding of the nascent chain to the RER membrane, but do not suggest a direct role for SSR in this process.

Precursor Translocation

Recent cross-linking studies, using both photo- and bifunctional cross-linking reagents, have indicated that SSRα can be crosslinked to both the mature region of secretory precursor translocation intermediates as well as to type I and type II integral membrane proteins (Krieg et al., 1989; Wiedmann et al., 1989; Gorlich et al., 1990; High et al., 1991). To determine if the apparent physical interaction between SSRα and the various translocation intermediates might reflect a direct functional contribution to translocation, the ΔSSR vesicles were assayed for preprolactin translocation activity. The experiment depicted in Fig. 5 compares the preprolactin processing activity of ΔSSR vesicles to that of
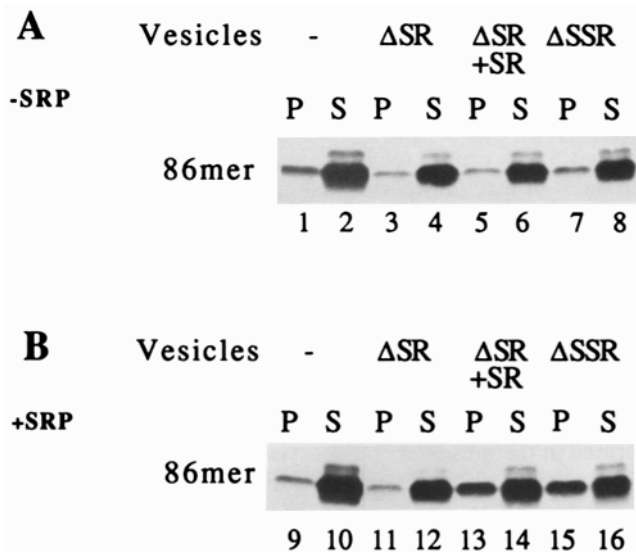
A

Vesicles   -   ΔSR   ΔSR +SR   ΔSSR

-SRP   P   S   P   S   P   S   P   S

86mer

1   2   3   4   5   6   7   8

B

Vesicles   -   ΔSR   ΔSR +SR   ΔSSR

+SRP   P   S   P   S   P   S   P   S

86mer

9   10   11   12   13   14   15   16

Figure 4. SRP-dependent cotranslational binding of preprolactin 86 mer to reconstituted vesicles. mRNA encoding the amino-terminal 86 amino acids of bovine preprolactin (86 mer) was translated in a wheat germ system in the absence (*top*) or in the presence (*bottom*) of 16 nM SRP. Where indicated, translations (25 μl) were supplemented with the various reconstituted vesicles at a concentration of 4 nmoles of phospholipids per reaction. After translation for 45 min at 25°C, reactions were chilled on ice, supplemented with EDTA to a final concentration of 25 mM, resolved into supernatant (*S*) and pellet (*P*) fractions by ultracentrifugation and analyzed by SDS-PAGE and autoradiography.

the ΔSR, ΔSR + SR, and CE vesicles. CE vesicles process preprolactin at ∼80% efficiency (Fig. 5, lane *4*) and of the processed prolactin 15% is protected from digestion by exogenous protease in the absence (lane *5*) but not the presence (lane *6*) of detergent. The ΔSR vesicles, containing, on average, 15% of the SR of CE vesicles, process preprolactin at ∼25% efficiency (lane *7*). As with the CE vesicles, a small, but significant, fraction of the mature prolactin is protected from digestion by exogenous protease (lane 8). The addition of SR prior to reconstitution of the ΔSR extract, provides full recovery of processing activity (lanes *10–12*). Perhaps most

vesicles   -   CE   ΔSR   ΔSR+SR   ΔSSR

PK   - + +   - + +   - + +   - + +   - + +
TX100   - - +   - - +   - - +   - - +   - - +

pPL —

PL —

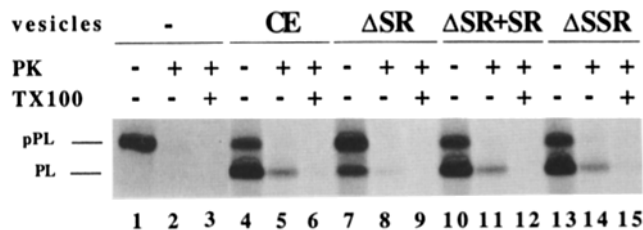1   2   3   4   5   6   7   8   9   10 11 12 13 14 15

Figure 5. Translocation activity of reconstituted vesicles: protease protection. mRNA encoding bovine preprolactin was translated in a rabbit reticulocyte lysate system in the presence of the indicated reconstituted vesicles, at a concentration of 8 nmoles of phospholipid per 25-μl reaction. After translation for 45 min at 25°C, samples were chilled on ice and mock incubated or incubated with proteinase K (*PK*) in the presence or absence of Triton X 100 (TK 100), as indicated, and analyzed by 12.5% SDS-PAGE and autoradiography. pPL and PL indicate the position of the precursor and mature form of prolactin, respectively.

significantly, the activity of the ΔSSR vesicles (lanes *13–15*) is indistinguishable from either the CE or ΔSR + SR vesicles, with 80% of the precursor processed to mature prolactin (lane *13*) and 15% of the prolactin protected from digestion by exogenous protease (lane *14*).

In mammalian microsomes, translocation and processing of preprolactin is strictly an SRP dependent process (Walter and Blobel, 1981). The SRP dependence of the processing reaction thus serves as a useful index of translocation activity. The preprolactin processing activity of the CE, ΔSR, ΔSR + SR, and ΔSSR vesicles, in the presence and absence of SRP, is shown in Fig. 6 *A*. The SRP-dependent arrest of preprolactin synthesis is depicted in lanes *1* and *2*. Under the described assay conditions, preprolactin synthesis is inhibited by ∼70–80% by addition of 16 nM SRP. In the absence of SRP, preprolactin processing activity of CE, ΔSR, ΔSR + SR, and ΔSSR vesicles is <10% of the total preprolactin (Fig. 6 *A*, compare lanes *3*, *5*, *7*, and *9*). In the presence of SRP, however, ∼70% of the preprolactin is processed to mature prolactin by CE, ΔSR + SR, and ΔSSR vesicles (compare lanes *4*, *8*, and *10* with lanes *3*, *7*, and *9*). Note that ΔSR vesicles process very little preprolactin in the presence or absence of SRP. This observation, although not unexpected, is significant in that it suggests that processing is occurring only upon translocation. If, for example, cleavage of the precursor was mediated by a population of signal peptidase complexes reconstituted to yield a cytoplasmically oriented active site, it would be expected that ΔSR vesicles would contain significant processing activity. In corroboration with the data depicted in Fig. 5, it is clear that depletion of SSR has little discernible effect upon the translocation of the secretory precursor, preprolactin. To further substantiate this conclusion, and to address the possibility that SSR functions in a catalytic manner, additional experiments were performed under conditions of limiting membrane concentration. Such conditions would be expected to unmask a kinetic contribution of SSR to translocation, assuming that SSR were to function proximal to an as yet hypothetical rate limiting step. In the experiment shown in Fig. 6 *B*, SRP-dependent processing was assayed at both low and high membrane concentrations (*1* and *6* nmol of vesicle phospholipid/25-μl reaction, respectively). The SRP-dependent inhibition of preprolactin synthesis is shown in lanes *1* and *2*. As is occasionally observed in the wheat germ system, a small amount of processing occurs in the absence of added membranes. This processing activity is lost upon further centrifugation of the wheat germ extract and thus is likely to originate from wheat germ derived membranes (data not shown). As depicted in Fig. 6 *A*, lanes *5* and *6*, a reduction in the concentration of CE vesicles to 1 nmol phospholipid/25-μl reaction results in a decrease of ∼50% in the SRP-dependent processing activity, indicating that the membranes are, in fact, present at limiting concentration. A similar reduction in SRP-dependent processing activity was observed when ΔSSR vesicles were assayed at limiting concentration (lanes *9* and *10*). These data suggest that SSR is unlikely to perform a catalytic function associated with translocation and further support the conclusion that SSR is not an essential component of the mammalian translocation apparatus.

Membrane Protein Integration

As noted previously, photocrosslinking experiments have

A

Vesicles	-		CE		ΔSR		ΔSR +SR		ΔSSR	
SRP	-	+	-	+	-	+	-	+	-	+

pPL—
PL—

    1   2   3   4   5   6   7   8   9   10

B

Vesicles	-		CE				ΔSSR			
Plipids (nmoles)	-		6		1		6		1	
SRP	-	+	-	+	-	+	-	+	-	+

pPL —
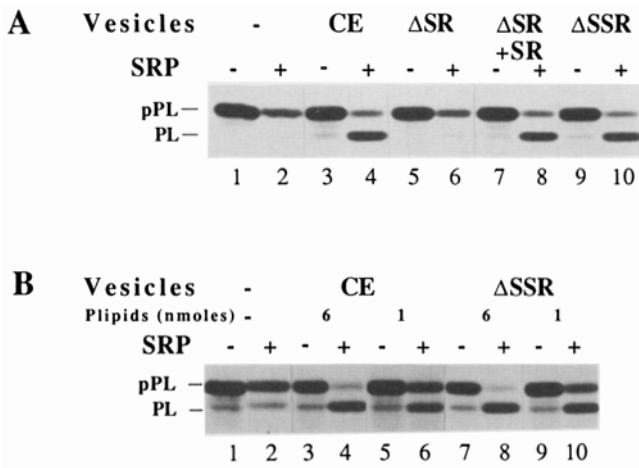PL —

    1   2   3   4   5   6   7   8   9   10

Figure 6. Translocation activity of reconstituted vesicles: SRP-dependent processing. Preprolactin mRNA was translated in a wheat germ system in the presence of the indicated vesicles at a concentration of either 4 nmole of phospholipids per 25 μl translation (*A*) or 1 and 6 nmole of phospholipids per 25 μl reaction (*B*). SRP (16 nM) was present during translation, as indicated. After a 45-min translation at 25°C, samples were precipitated by 10% TCA and analyzed by 12.5% SDS-PAGE and autoradiography. pPL and PL indicate the position of preprolactin and mature prolactin, respectively.

demonstrated that SSRα resides in physical proximity to both type I and type II membrane protein translocation intermediates (High et al., 1991; Thrift et al., 1991). To ascertain if depletion of SSR disrupted the process of membrane protein integration, the membrane protein integration activity of RMek, CE, ΔSR, ΔSR + SR, and ΔSSR vesicles was determined with respect to the Sindbis EI glycoprotein, a type I membrane protein. Cotranslational assembly of E1 into RMek yields two slower migrating forms, g1E1 and g2E1,

which arise through addition of either one or two core oligosaccharides, respectively (Fig. 7 *A*, lane *4*). A population of E1 of slightly slower mobility is occasionally observed and is comprised of glucosylated g1E1 and g2E1. The mobility differences between the glucosylated and deglucosylated forms can be seen by comparison of the translation products of reactions performed in the presence (lane *3*), or absence (lane *4*), of 1-deoxynojirimycin, an inhibitor of glucosidase I and II. Addition of a glycosylation acceptor tripeptide inhibits glycosylation, allowing detection of the processed, or cleaved, form of E1 (Fig. 7 *A*, lane *5*). E1 was cotranslationally assembled into RMek, CE, ΔSR, ΔSR + SR, and ΔSSR vesicles, subjected to urea extraction, and integrated E1 separated from soluble E1 by sedimentation. To simplify interpretation of the data, translations were performed in the presence of glycosylation acceptor tripeptide. Under these conditions, the majority of the E1 translation products are recovered as either the precursor (pE1) or mature (uE1) forms. In the absence of vesicles (Fig. 7 *B*, lanes *6* and *7*), pE1 is recovered almost entirely in the supernatant fraction. In the presence of RMek, pE1 is cleaved to yield the mature form (uE1) which sediments predominantly (~70%) in the pellet fraction (Fig. 7, lane *8* vs lane *9*). CE vesicles behave quite similarly to RMek, with the majority of the uE1 sedimenting in the pellet fraction (Fig. 7, lanes *10* and *11*). ΔSR vesicles only support limited cleavage or integration of pE1, as the majority of the pE1 was recovered in the supernatant fraction (Fig. 7, lanes *12* and *13*). ΔSR + SR vesicles display none of the functional defects evident in the ΔSR vesicles and support both efficient cleavage and integration of pE1 (lanes *14* and *15*). ΔSSR vesicles also efficiently process and integrate pE1, with the majority of the uE1 sedimenting with the pellet fraction (Fig. 7, lanes *16* and *17*). On the basis of these experiments, it appears that SSR is not required for the integration of membrane proteins. Although glycosylation acceptor peptide was present in the experi-
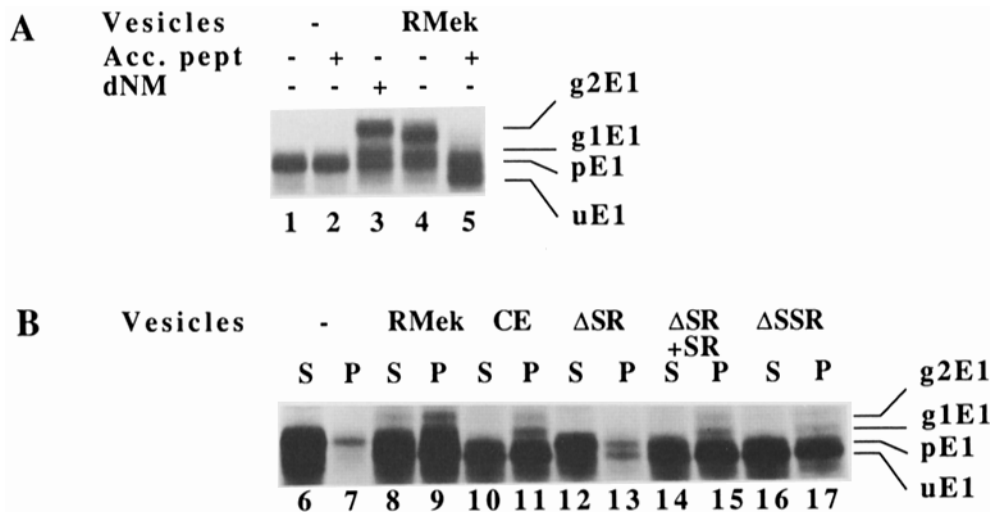
A

Vesicles	-		RMek		
Acc. pept	-	+	-	-	+
dNM	-	-	+	-	-

                                g2E1
                                g1E1
                                pE1
    1   2   3   4   5   uE1

B

Vesicles	-		RMek		CE		ΔSR		ΔSR +SR		ΔSSR	
	S	P	S	P	S	P	S	P	S	P	S	P

                                                            g2E1
                                                            g1E1
                                                            pE1
    6   7   8   9   10 11 12 13 14 15 16 17   uE1

Figure 7. Translocation activity of reconstituted vesicles: integration of membrane proteins. mRNA encoding Sindbis E1 glycoprotein was translated in a reticulocyte lysate system in the presence of salt-EDTA washed rough microsomes (*RMek*) or the indicated reconstituted vesicles, at a concentration of 6 nmoles of phospholipids per 25-μl reaction. Where indicated the glycosylation acceptor tripeptide *N*-acetyl Asn-Leu-Thr-amide (Acc. pept) or the glucosidase I and II inhibitor 1-deoxynojirimycin (*dNM*) were present during translation at concentration of 100 μmolar. After translation for 45 min at 25°C, samples were chilled on ice, and either prepared directly for gel analysis (lanes *1* to *5*) or extracted with urea, separated by ultracentrifugation into a supernatant (*S*) and pellet (*P*) fractions, and analyzed by 9% SDS-PAGE and autoradiography. pE1 and uE1 denote the position of the precursor and the processed, unglycosylated E1 protein, respectively. g1E1 and g2E1 denote the position of the processed E1 protein containing 1 and 2 N-linked oligosaccharide chains, respectively. Note that in the presence of dNM and RMek, both 1gE1 and 2gE1 have a slightly retarded migration on the gel, due to the presence of the three terminal glucose residues on the oligosaccharide chains.

ments depicted in Fig. 7, the presence of small amounts of glE1 and g2E1 forms indicate that RMek, CE, ΔSR + SR, ΔSSR, but not ΔSR, vesicles are capable of glycosylation of the E1 precursor. As expected, the glE1 and g2E1 forms are integrated in the membranes and sediment in the pellet. In general, however, reconstituted membranes have only a fraction of the oligosaccharyl transferase activity of native membranes.

Discussion

In this communication we report the results of studies in which two resident membrane protein components of the RER, the SRP receptor (SR), and the SSR, were depleted from detergent solubilized rough microsomal membranes and the functional contribution of the two components to protein translocation determined in reconstituted vesicles. Depletion was accomplished through use of antibody supports prepared from affinity purified IgG fractions. With antibodies of sufficient specificity and affinity, this method allows precise manipulation of the protein composition of the detergent extract. It is expected that the described experimental paradigm will aid in the functional analysis of other, previously identified resident RER proteins, such as the signal peptidase complex (Evans et al., 1986; Baker and Lively, 1987) and the ribophorins (Kreibich et al., 1978a,b) as well as in the characterization of novel components.

Data obtained in studies of the activity of vesicles prepared from SR-depleted extracts (ΔSR), as well as vesicles prepared from depleted extracts supplemented with immunoaffinity purified SR (ΔSR + SR), provide additional direct experimental support for the proposed function of SR in the targeting of the ribosome/nascent chain/SRP complex to the RER membrane (Meyer and Dobberstein, 1980a,b; Gilmore and Blobel, 1982a,b; Meyer et al., 1982). Thus, ΔSR vesicles, although functional in assays of ribosome binding, display little or no activity with respect to elongation arrest release, SRP-dependent precursor targeting or secretory and membrane protein translocation. The described functional defects arise entirely through depletion of SR; full complementation is obtained by reconstitution of the immunoaffinity purified SR with the depleted extract. These observations are of interest for two reasons. Firstly, they demonstrate that a protein component of rough microsomes can be reversibly depleted from detergent solubilized membranes and its function determined in reconstituted vesicles. Secondly, with the SR depletion experiments serving as a positive control, these results indicate that biochemical depletion and reconstitution procedures can be used with confidence to assess the functional contribution, to translocation, of other RER membrane proteins.

In contrast to the results obtained with ΔSR vesicles, depletion of SSR was without effect on microsomal translocation activity. In view of the wealth of cross-linking data indicating the physical proximity of SSRα, during translocation, to both secretory and membrane protein precursors, these results were unexpected (Wiedmann et al., 1987; Krieg et al., 1989; Wiedmann et al., 1989; Görlich et al., 1990; High et al., 1991; Thrift et al., 1991). Although physical proximity does not definitively indicate a functional interaction, it is nonetheless difficult to rationalize the contradictory conclusions which must be drawn from the two different experimental approaches. At present, the primary evidence supporting a direct functional role for SSRα in translocation is the demonstration that the translocation and elongation arrest release activity of rough microsomes can be blocked by treatment of membranes with IgG or Fab fragments directed against the cytoplasmic domain of the protein (Hartmann et al., 1989). These results have been suggested to indicate that either SR and SSR are functionally linked, or that inhibition of signal sequence receptor activity causes a kinetic block and subsequent inhibition of elongation arrest release (Hartmann et al., 1989). Although such data are consistent with the proposal that SSR functions as a signal sequence receptor, they are not unique to SSR. IgG and Fab fragments directed against the cytoplasmic domain of ribophorin I, for example, also inhibit both the translocation and elongation arrest release activity of rough microsomes (Yu et al., 1990). Ribophorin I was not, however, identified by the photocrosslinking approach as a component residing in physical proximity to the nascent chain (Krieg et al., 1989; Wiedmann et al., 1989). It would appear, therefore, that either the photocrosslinking approach provides a biased sampling of the protein components adjacent to the nascent chain or that antibody inhibition studies may be of limited usefulness in defining the activity of integral membrane proteins.

On the assumption of the predicted, nonselective reactivity of photosensitive cross-linking reagents, incorporation of such derivatives into the nascent chain should allow a direct analysis of the protein components residing in proximity to the nascent chain (Wiedmann et al., 1987, 1989; Krieg et al., 1989). It is possible, however, that membrane components could exhibit substantial differences in reactivity to these reagents, a phenomenon which would yield an inaccurate map of the protein environment surrounding the nascent chain. It is noteworthy, for example, that the β subunit of SSR, which is found in a stable, 1:1 stoichiometric association with the α subunit, is not labeled by photocrosslinking (Wiedmann et al., 1987, 1989; Krieg et al., 1989; Görlich et al., 1989). SSRβ can, though, be crosslinked to SSRα with the bifunctional cross-linking reagent dithiobis(succinimidyl propionate) (DSP) (Görlich et al., 1990). If the protein environment of the nascent chain is determined with the chemical cross-linking reagent disuccinimidyl suberate (DSS), both secretory and membrane protein precursors are crosslinked to a 34-kD protein, which is distinct from SSRα (Kellaris et al., 1991). If, however, crosslinking of translocation intermediates is performed with m-maleimidobenzoyl-sulfosuccinimidoester (sulfo-MBS) or carbodimide reagents, crosslinks between translocation intermediates and with SSRα are seen (Görlich et al., 1990). Finally, photocrosslinking with a signal-anchor type membrane protein yields crosslinks to RER membrane proteins of 37 and 42 kD (High et al., 1991). These data suggest that the protein environment surrounding the nascent chain may be far more complex than previously expected (Krieg et al., 1989) and that the 34-kD membrane protein previously identified as SSRα may be one of a population of proteins of similar molecular weight that are in physical proximity to the nascent chain and display differential reactivity to various chemical cross-linking reagents. It would appear, therefore, that the precise identification of the signal sequence receptor, or perhaps signal sequence binding component of a protein conducting channel (Simon and Blobel, 1991) must await further studies.

Both SSR and ribophorin I individually constitute ~1% of the total microsomal protein (Marcantonio et al., 1984; Hartmann et al., 1989). It has been estimated that saturation of either SSR or ribophorin I with IgG or Fab fragments should not, therefore, contribute significantly to the available membrane surface area (Hartmann et al., 1989; Yu et al., 1990). Such calculations suggest that the probability of an antibody dependent, non-specific steric disruption of translocation is low and further substantiate the conclusion that SSR and ribophorin I are in close proximity to translocation sites. Chemical cross-linking studies provide additional, albeit indirect, support for this conclusion, as ribophorin I, as well as SSR, are members of a subset of RER proteins which can be crosslinked to the membrane-bound ribosome (Kreibich et al., 1978b; Collins and Gilmore, 1991). Thus, both crosslinking and antibody inhibition studies provide useful indications of the protein components which are in physical proximity to the translocating nascent chain. Members of this subset of RER proteins may, however, perform functions related to, but functionally distinct from, translocation. Possible functions may include posttranslational modifications, membrane protein assembly and/or proteolytic degradation.

SSR is a relatively abundant component of the RER membrane (Hartmann et al., 1989). Because of its high enrichment, it can be argued that the levels of depletion achieved in this study (>95%) are insufficient to remove SSR activity, if it is assumed that SSR functions catalytically. SSR is present in RM in nearly stoichiometric amounts to bound ribosomes and on the basis of such a relationship it has been suggested that SSR may function as, or be part of, a translocation pore (Krieg et al., 1989; Wiedmann et al., 1989; Görlich et al., 1990). If this is indeed the case, SSR would not be expected to function in a catalytic manner. Conjecture aside, however, nearly complete depletion of a catalytic activity would be evident as a kinetic effect. As example, and as demonstrated herein, partial depletion of SR, a membrane component which does function in a catalytic manner, markedly inhibits translocation. When ΔSSR membranes are assayed under conditions where the membrane concentration is limiting, however, no kinetic effects on translocation were observed.

In conclusion, as defined in biochemical reconstitution assays, the RER membrane protein identified as SSR is unlikely to serve an essential function in translocation. It remains possible, however, that functionally redundant proteins are present in RM and although unsusceptible to crosslinking by current procedures, fully complement the activities proposed for SSR.

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