

# The $\beta$ Subunit of the Signal Recognition Particle Receptor Is a Transmembrane GTPase that Anchors the $\alpha$ Subunit, a Peripheral Membrane GTPase, to the Endoplasmic Reticulum Membrane

Joshua D. Miller, Shoji Tajima, Leander Lauffer, and Peter Walter

Department of Biochemistry and Biophysics, University of California Medical School, San Francisco, California 94143-0448

**Abstract.** The signal recognition particle receptor (SR) is required for the cotranslational targeting of both secretory and membrane proteins to the endoplasmic reticulum (ER) membrane. During targeting, the SR interacts with the signal recognition particle (SRP) which is bound to the signal sequence of the nascent protein chain. This interaction catalyzes the GTP-dependent transfer of the nascent chain from SRP to the protein translocation apparatus in the ER membrane. The SR is a heterodimeric protein comprised of a 69-kD subunit (SR $\alpha$ ) and a 30-kD subunit (SR $\beta$ ) which are associated with the ER membrane in an unknown manner. SR $\alpha$  and the 54-kD subunit of SRP (SRP54) each contain related GTPase domains which are required for SR and SRP function. Molecular cloning and sequencing of a cDNA encoding SR $\beta$

revealed that SR $\beta$  is a transmembrane protein and, like SR $\alpha$  and SRP54, is a member of the GTPase superfamily. Although SR $\beta$  defines its own GTPase subfamily, it is distantly related to ARF and Sar1. Using UV cross-linking, we confirm that SR $\beta$  binds GTP specifically. Proteolytic digestion experiments show that SR $\alpha$  is required for the interaction of SRP with SR. SR $\alpha$  appears to be peripherally associated with the ER membrane, and we suggest that SR $\beta$ , as an integral membrane protein, mediates the membrane association of SR $\alpha$ . The discovery of its guanine nucleotide-binding domain, however, makes it likely that its role is more complex than that of a passive anchor for SR $\alpha$ . These findings suggest that a cascade of three directly interacting GTPases functions during protein targeting to the ER membrane.

**I**N eucaryotic cells the translocation of most secretory and the integration of most membrane proteins into the endoplasmic reticulum (ER) are cotranslational events. Targeting of ribosomes synthesizing such proteins from the cytoplasm to the ER is catalyzed by the signal recognition particle (SRP),<sup>1</sup> which binds to signal sequences on the polypeptide chains emerging from the ribosome (reviewed in Walter and Johnson, 1994). Subsequent to signal sequence recognition in the cytosol, the resulting complex is targeted to the cytoplasmic face of the ER membrane via the interaction of SRP with its membrane bound receptor (Gilmore et al., 1982a,b; Meyer et al., 1982). Upon binding to the SRP receptor (SR), SRP dissociates from both the signal se-

quence and the ribosome, allowing the engagement of the ribosome with the translocon, a protein apparatus in the membrane that forms a pore through which the nascent polypeptide moves across the lipid bilayer (Gilmore and Blobel, 1983; Simon and Blobel, 1992; Görlich and Rapoport, 1993; Crowley et al., 1994). Thus, SRP and SR are the "initiation factors" of protein translocation mediating both targeting and the formation of the ribosome/translocon junction.

Both SR and SRP are complex structures: SRP is a ribonucleoprotein consisting of six distinct protein subunits and one RNA subunit (Walter and Blobel, 1980; Walter and Blobel, 1982). The most phylogenetically conserved SRP protein subunit, SRP54, contains the signal sequence-binding site of SRP and mediates SRP binding to SR (Krieg et al., 1986; Kurzchalia et al., 1986; Miller et al., 1993; Brown et al., 1994). The SR is a heterodimeric membrane protein consisting of a 69-kD (SR $\alpha$ ) and a 30-kD subunit (SR $\beta$ ) (Tajima et al., 1986). It is now known how SR interacts with either SRP54 or the membrane of the ER.

GTP is required for multiple steps of the targeting reaction, and both SR $\alpha$  and SRP54 contain GTPase domains (Connolly and Gilmore, 1986, 1989; Bernstein et al., 1989; Römisch et al., 1989). The GTPase domains of SR $\alpha$  and SRP54 are related and define a new subfamily in the GTPase

Dr. Tajima's current address is Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565, Japan.

Dr. Lauffer's present address is Behringwerke AG, Postfach 1140, D-35001 Marburg, Germany.

Address all correspondence to Peter Walter, Dept. of Biochemistry and Biophysics, University of California Medical School, San Francisco, CA 94143-0448. Tel.: (415) 476-5017. Fax: (415) 476-5233.

1. *Abbreviations used in this paper:* DIFP, diisopropylfluorophosphate; DTT, dithiothreitol; KOAc, potassium acetate; Mg(OAc)<sub>2</sub>, magnesium acetate; SRP, signal recognition particle; SR, SRP receptor; TEA, triethanolamine.

superfamily (Bernstein et al., 1989; Römisch et al., 1989; Bourne et al., 1991). A model describing the cycle of GTP binding and hydrolysis by SRP54 during the protein targeting reaction has been proposed (Miller et al., 1993). According to this model, SRP54 becomes stabilized in a nucleotide-free state when SRP binds to a signal sequence exposed on the ribosome. Interaction of SRP with the SR on the ER membrane in the presence of translocon components stimulates GTP binding to SRP54 with the concomitant release of SRP from the signal sequence and ribosome, which then engage with the translocon to translocate the protein across the membrane. After release from the ribosome and signal sequence, the SRP, in its GTP-bound state, remains tightly bound to the SR. The SR then functions as a GTPase activating protein for SRP54 and, upon hydrolysis of the bound GTP, SRP is released from SR into the cytosol, free to enter into another round of targeting. SR and SRP work catalytically to promote the interaction of the nascent chain-ribosome complex with the translocon and do not remain associated after the ribosome/translocon junction is formed. Thus, during protein targeting the assembly and disassembly of complexes is regulated by GTP binding proteins, as are other protein-protein interactions that need to be formed and broken in cells in a coordinated and regulated manner (Bourne et al., 1990).

The individual contribution that the two subunits make to SR function is largely unknown. There is evidence that GTP binding to SR $\alpha$  is required for translocation, but it is not known what role this serves (Rapiejko and Gilmore, 1992). SR $\beta$  has no known function. Either one or both subunits could conceivably be required for any of the known SR functions of SRP binding, membrane binding, regulation of the SRP54 GTPase cycle, and promoting translocation. Here, we examine the role of the two subunits in the association of SR with SRP and with the ER membrane. We also show that, like SR $\alpha$  and SRP54, SR $\beta$  is a member of the GTPase superfamily. This brings to three the number of directly interacting GTPases, suggesting that a GTPase cascade of unprecedented complexity functions during protein targeting.

## Materials and Methods

### Materials

$\alpha$ -[ $^{32}$ P]GTP (3,000 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL); Na[ $^{125}$ 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, cyanogen bromide (CNBr) 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); DEAE Affigel Blue and hydroxylapatite from Bio-Rad Laboratories (Richmond, CA).

### General Methods

Preparation of rough microsomal membranes, their salt extraction and purification of SRP and SRP receptor were performed as described previously (Gilmore and Blobel, 1983; Walter and Blobel, 1983a,b; Tajima et al., 1986). Immunoblotting was performed using  $^{125}$ I-labeled secondary antibodies as previously described (Tajima et al., 1986). SR $\alpha$  was detected with the mouse monoclonal IgG antibody directed against epitope A

(Tajima et al., 1986), mp30 with a rabbit polyclonal serum (Tajima et al., 1986), and SR $\beta$  with a mouse monoclonal antibody described here.

### Preparation of Monoclonal Antibody to SR $\beta$

The anti-SR $\beta$  antibody is an IgM made by injecting Freund's adjuvant emulsified SR $\beta$  (purified by preparative SDS-PAGE) into the foot pad of a mouse followed by dissection of the popliteal lymph node and fusion to myeloma cells to create a hybridoma cell line. Hybridoma cells were propagated as ascites tumors. The monoclonal antibody was identified as an IgM using a kit purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). IgM was purified from mouse ascites fluid. To this end, the IgM was bound to anti-mouse IgM-Sepharose, washed with 0.5 M sodium chloride/10 mM phosphate buffer, pH 7.5, 0.1% Triton X-100 and eluted with 3.5 M magnesium chloride.

### Alkaline Extraction of Microsomal Membranes

Three different solutions were used for alkaline extraction: (a) 100 mM sodium carbonate, pH unadjusted (pH 11.2); (b) 100 mM sodium carbonate, adjusted to pH 12.0 by the addition of sodium hydroxide; and (c) 100 mM sodium hydroxide, pH 13.0. Membranes were diluted 1:100 into alkaline solution to obtain a final membrane concentration of 0.04 equivalents (eq)/ml (see Walter and Blobel, 1983a) for definition of equivalent). After 30 min at 25°C, the reactions were centrifuged for 30 min at 100,000 rpm in a Beckman TL 100.1 rotor. Supernatant and pellet fractions were analyzed by SDS-PAGE and immunoblotting.

### Triton X-114 Extraction of Microsomal Membranes

Membranes were solubilized at 0.3 eq/ $\mu$ l in 1% Triton X-114, 10 mM Tris-HCl, pH 7.5, 150 mM sodium chloride, and 1 mM dithiothreitol (DTT). After incubation on ice for 15 min, the reactions were transferred to a 37°C water bath for 3 min to induce phase separation. Detergent-poor and detergent-rich phases were separated by a 5-min centrifugation in a microfuge through a cushion of 175 mM sucrose in the above buffer containing 0.06% Triton X-114.

### Trypsin Treatment of Microsomal Membranes

Salt-extracted microsomal membranes were diluted to 2 eq/ $\mu$ l in high-salt buffer containing 50 mM triethanolamine (TEA), pH 7.5, 500 mM potassium acetate (KOAc), 5.5 mM magnesium acetate (Mg(OAc) $_2$ ), 0.5 mM (ethylenedinitrilo)tetraacetic acid (EDTA), 1 mM DTT. Trypsin-TPCK was added and the reaction was incubated on ice for 1 h. Digestion was stopped by addition of 2 mM diisopropylfluorophosphate (DIFP), 1 mM PMSF and 100 U/ml Trasylol. After 15 min on ice the membranes were either assayed as in Fig. 1 B, or pelleted by centrifugation at 50,000 rpm in a Beckman Ti 70.1 rotor for 30 min through a cushion of 250 mM sucrose in high-salt buffer containing 0.1 mM PMSF. The membrane pellet was resuspended in high-salt buffer and the centrifugation was repeated. After this washing step, the pellet was dissolved in 50 mM TEA, 250 mM sucrose, and 1 mM DTT. The membrane suspension was frozen in liquid nitrogen and stored at -80°C until further use.

### SRP-Sepharose Chromatography

Trypsinized membranes were diluted to 1 eq/ $\mu$ l in 1% Nikkol, 50 mM TEA, pH 7.5, 375 mM KOAc, 250 mM sucrose, 1 mM DTT, 10 U/ml Trasylol, 0.5 mM PMSF, and 0.1 mM DIFP and were extracted for 15 min on ice. The soluble fraction was obtained as the supernatant after a 30-min centrifugation at 100,000 rpm in the Beckman TL 100.1 rotor. The solubilized membranes were adjusted to 0.13 eq/ $\mu$ l in equilibration buffer (50 mM TEA, pH 7.5, 50 mM KOAc, 5 mM Mg(OAc) $_2$ , 250 mM sucrose, 1 mM DTT and 0.5% Nikkol) and 650  $\mu$ l was applied to a 0.15 ml SRP-Sepharose column containing 0.15 mg of covalently coupled SRP. After washing with 0.6 ml of equilibration buffer, the column was eluted with 0.8 ml elution buffer (50 mM TEA, pH 7.5, 10 mM KOAc, 25 mM Mg(OAc) $_2$ , 250 mM sucrose, 1 mM DTT, and 0.5% Nikkol). Coupling of SRP to CNBr-Sepharose was as described previously (Tajima et al., 1986).

### Protein Sequencing

Immunopurified SR (Tajima et al., 1986) was adjusted to 5% twice recrystallized SDS and heated to 80°C for 10 min to dissociate the two subunits and then fractionated by reverse phase chromatography on an Alltech C4 column using an IBM HPLC to yield purified SR $\alpha$  and SR $\beta$ .

Peptide sequence of SR $\beta$  was determined by Edman degradation, using an ABI automated sequenator. Sequencing the intact protein yielded two identical, overlapping sequences that were staggered by two amino acids: XXMGDGGGVGGAFQPYLDSLRL and XXXXMGDGGGVGGAFQPYLDSLRL. The yield of PTH amino acid released/ $\mu$ g per cycle of Edman degradation was considerably lower than that obtained from proteolytic fragments. Therefore, it is likely that the true amino terminus is blocked and that we obtained sequence from a small amount of proteolytic breakdown product. We also sequenced the amino-terminus of a proteolytic fragment that was generated by lysyl-endopeptidase digestion of SR $\beta$  and purified on a microbore C18 reverse phase column from Vydac using a Rainin HPLC. The sequence read KWLAK.

A third peptide sequence was obtained by performing five rounds of Edman degradation on total CNBr cleaved SR $\beta$  to expose a proline residue at the amino-terminus of one of the CNBr fragments. The amino-termini of all the other CNBr fragments were then blocked with *ortho*-phthalaldehyde (OPA; Brauer et al., 1984). Because proline does not react with OPA, it remains unblocked and, therefore, susceptible to Edman degradation. Sequencing was then resumed yielding a single sequence from the CNBr fragment. The sequence read PLIACNKQD.

### cDNA Cloning

To obtain a cDNA clone of SR $\beta$ , a Madin-Darby Canine Kidney (MDCK) cell cDNA library constructed in the plasmid vector pEX (Stanley and Luzzio, 1984) was screened using the anti-SR $\beta$  monoclonal described here. A total of  $3 \times 10^5$  bacterial colonies were screened by a modification of the colony blotting procedure of Stanley (Stanley, 1983). Expression of the cDNAs was induced by incubating the filters at 42°C, lysing the cells at 90°C in 5% SDS, probing with the monoclonal antibody and using an alkaline phosphatase-conjugated secondary antibody to detect positive colonies. Four positive colonies passed secondary and tertiary screening; these cDNA clones were partially sequenced using the double-stranded Sequenase (USB) protocol and identified as encoding SR $\beta$  sequences because their predicted translation products contained the amino acid sequence obtained from direct protein sequencing of the amino-terminus. They were then subcloned into a Bluescript-II vector (Stratagene Inc., La Jolla, CA), single-stranded DNA was synthesized and the entire cDNAs were sequenced on both strands using Sequenase. The predicted translation products of both clones contained both additional peptide sequences derived from sequencing SR $\beta$ , thus confirming the identity of the clones.

The predicted translation products of both clones contained consensus sequences for GTP-binding proteins (Bourne et al., 1991). Two sets of clones (1.0 and 1.2 kb in length) were isolated; they differed from each other by the spacing between region G-1 and region G-3 of the GTP-binding consensus sequence, by the length of the 3' poly(A) tail, and by the overall length of the cDNA insert. The 1.0 kb clone presumably encodes SR $\beta$ . It contains an ~60 nucleotide-long poly(A) tail, and the spacing between regions G-1 and G-3 of the predicted protein product conforms to the spacing found in other GTPases. In contrast, the 1.2 kb clone contains only six A residues at the polyadenylation site followed another 500 bp of noncoding sequence. This clone also contains an in frame deletion of 26 amino acids between regions G-1 and G-3 (residues 82-107 from the SR $\beta$  sequence shown in Figs. 3 and 4 would be deleted). We consider it likely that this cDNA clone was derived from an alternately or erroneously spliced form of the mRNA.

The amino acid sequence deduced from the canine cDNA does not begin with a methionine and extends just past the amino acid sequence derived from amino-terminal sequencing. Therefore, a full-length SR $\beta$  cDNA was isolated by screening a murine teratocarcinoma cDNA library constructed

Table I. Comparison of Canine and Murine Proteins

Amino acid no.	7	9	12'	12"	13	39	42	56	59	67	72	86	100
Canine SR $\beta$	P	M	G	G	V	V	V	R	R	L	N	L	M
Murine SR $\beta$	R	V			A	A	L	W	K	F	D	Q	I
Amino acid no.	102	106	108	109	124	126	128	132	137	166	170	173	183
Canine SR $\beta$	R	T	A	T	L	F	E	A	I	S	T	F	T
Murine SR $\beta$	K	N	G	N	F	L	D	S	V	A	S	L	A

The positions where the two sequences differ are indicated. The amino acid numbers refer to the murine protein as shown in Fig. 3 A. In the canine sequence there is an insertion of GG, labeled 12' and 12", between amino acids 12 and 13 of the murine protein.

in  $\lambda$ -ZAP (Stratagene) using the canine cDNA as a hybridization probe (Maniatis et al., 1982). Eight independent clones were obtained from  $1.2 \times 10^6$  plaques screened and were verified by DNA sequencing. All of the murine clones corresponded to the 1 kb canine clones described above. The differences between the predicted canine and murine proteins are listed in Table I.

### Sequence Analyses

Homology searches were conducted using the BLAST network at the NCBI (Altschul et al., 1990). The *Saccharomyces cerevisiae* SR $\beta$  homologue was found as a predicted open reading frame in genomic sequence from chromosome 11 (accession no. Z66877). Limited sequence similarity was also found with the *S. cerevisiae* IRA2 protein (accession no. RBY12; residues 191-218 of murine SR $\beta$  are 59% identical to residues 439-466 of *S. cerevisiae* Ira2p) and the *S. cerevisiae* SWI4 protein (accession no. S07106; residues 164-228 of murine SR $\beta$  are 33% identical to residues 800-864 of *S. cerevisiae* Swi4p); however, in both cases the similarities were not phylogenetically conserved between mammalian and yeast SR $\beta$  sequences and are therefore unlikely to be functionally significant. Secondary structure predictions based on amino acid sequence were done using Chou-Fasman parameters (Rawlings et al., 1983).

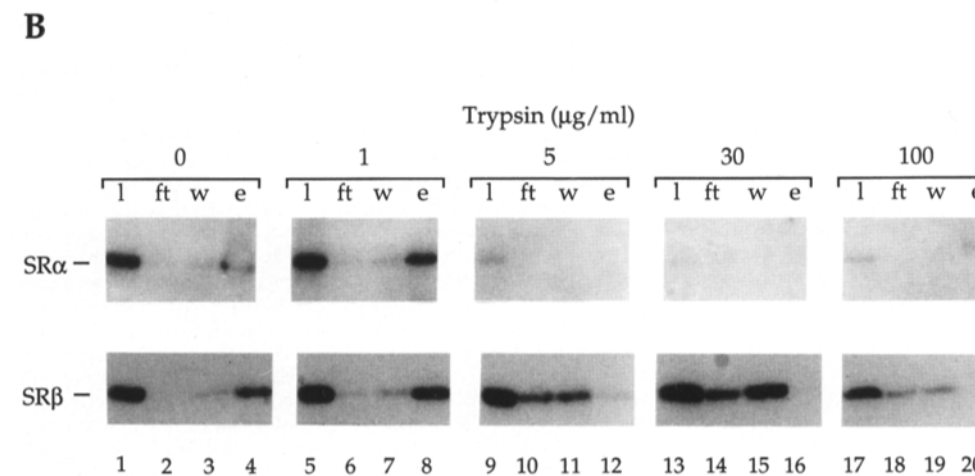
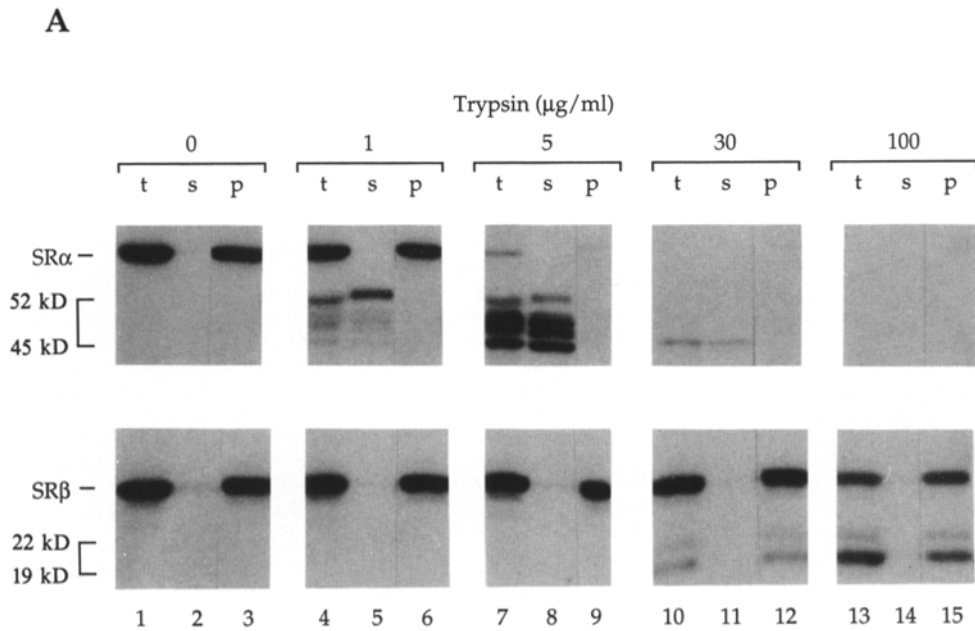
### GTP Cross-linking Assay

SRP-Sepharose purified SR (Gilmore and Blobel, 1983; Tajima et al., 1986) was mixed at 20 nM with 0.3  $\mu$ M  $\alpha$ - $^{32}$ P]-labeled GTP at 25°C in 50 mM TEA, pH 7.5, 150 mM KOAc, 5 mM Mg(OAc)<sub>2</sub>, 1 mM DTT, and 0.5% Nikkol. Some reactions were supplemented with unlabeled nucleotide to compete for binding with the radiolabeled substrate. After a 20-min incubation at 25°C the 20- $\mu$ l reactions were placed in plastic weigh boats on ice and UV irradiated (6 cm from a 6000 W/cm<sup>2</sup> UV source) for 5 min to form covalent cross-links of the bound radiolabeled nucleotide to the protein (Nath et al., 1985; Miller et al., 1993). The reactions were then precipitated with an equal volume of 30% trichloroacetic acid to remove uncrosslinked radiolabeled nucleotide and analyzed by SDS-PAGE followed by autoradiography. Quantitation was done using a Bio-Rad densitometer to scan autoradiograms that were determined to be in the linear range of both the film and the machine. Data points are experimental and the line is generated as a best fit to the equation:  $B = B_{\max} (1 - [I]/([I] + K_i(1 + ([S]/K_D))))$  (a modification of equation III-5 described by Segel [1975]) using the program Kaleidagraph (Abelbeck Software, 1989) on a Macintosh II computer. B, amount of  $\alpha$ - $^{32}$ P]GTP cross-linked to SRP54; B<sub>max</sub>, amount of a  $\alpha$ - $^{32}$ P]GTP crosslinked to SRP54 in the absence of competitor; [I], concentration of competitor; K<sub>i</sub>, dissociation constant of competitor; K<sub>D</sub>, dissociation constant of  $\alpha$ - $^{32}$ P]GTP; [S], concentration of  $\alpha$ - $^{32}$ P]GTP. GTP hydrolysis was measured in these reactions as described previously (Miller et al., 1993) and found to be negligible (not shown).

### Results

#### SR $\alpha$ Is Required for SR Binding to SRP

To investigate the individual roles of the two SR subunits in the interaction with SRP, we took advantage of the different sensitivities of SR $\alpha$  and SR $\beta$  to proteolytic digestion. As shown in Fig. 1 A, trypsin at a concentration as low as 1  $\mu$ g/ml begins to degrade SR $\alpha$  (lanes 4-6), while a minimal concentration of 30  $\mu$ g/ml is required to begin to degrade SR $\beta$  (lanes 10-12). To generate extracts containing different relative amounts of SR $\alpha$  and SR $\beta$ , we first incubated rough microsomal membrane vesicles with variable amounts of trypsin, extracted peripherally attached digestion products with high salt and then solubilized the remaining membrane-associated SR fragments with detergent. Passing a detergent extract prepared from undigested membranes over an SRP-Sepharose affinity column resulted in the quantitative binding of the SR $\alpha$ /SR $\beta$  complex to the resin (Fig. 1 B, lanes 1-3) and allowed the recovery of the bound receptor by elution (Fig. 1 B, lane 4). This result was expected, as SR was originally purified by a similar procedure using affinity chro-



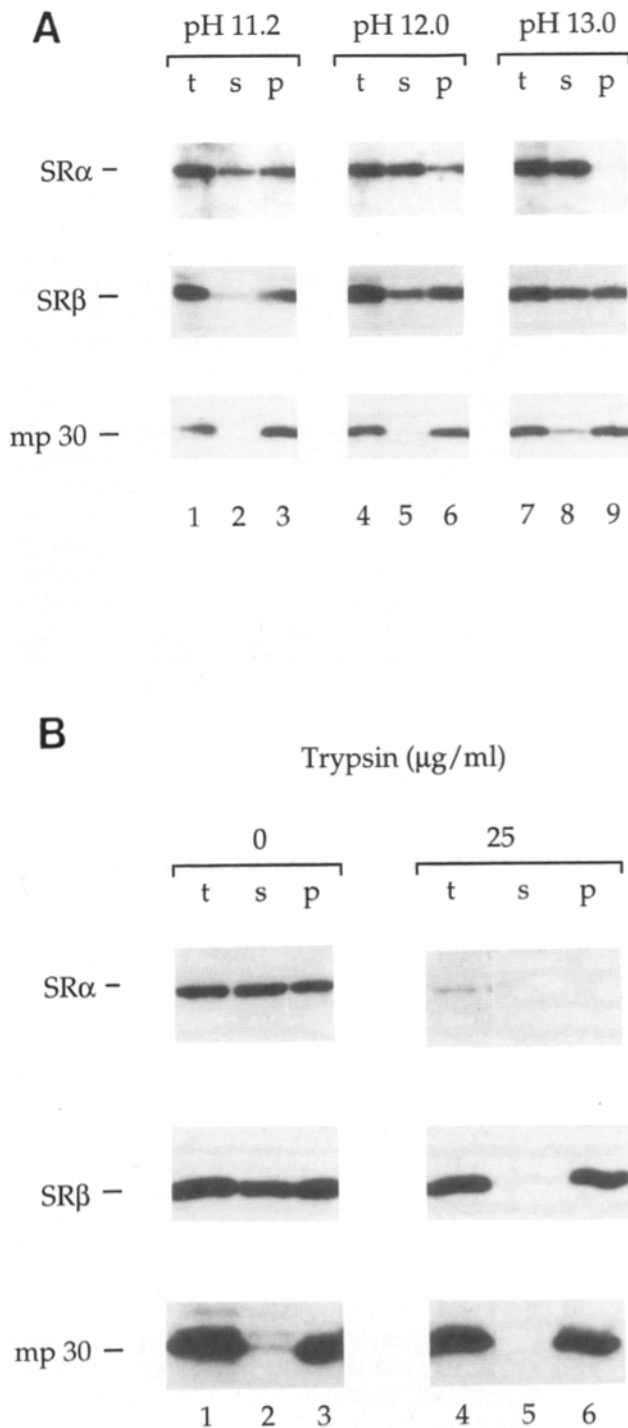
**Figure 1.** Requirement for intact SR $\alpha$  for SRP binding. **(A)** tryptic digestion pattern of SR. Canine microsomal membranes were digested in high salt (500 mM potassium) with the indicated concentration of trypsin-TPCK. Proteolysis was stopped by the addition of protease inhibitors and the membranes were pelleted by centrifugation. Equivalent amounts of the total reaction mixture (*t*), and of the supernatant (*s*) and pellet fractions (*p*) were separated by SDS-PAGE and immunoblotted for SR $\alpha$  and SR $\beta$  as indicated. The position of undigested protein is labeled and the position of proteolytic breakdown products are indicated by brackets. **(B)** Binding of proteolyzed SR to SRP-Sepharose. The trypsinized membranes from **A** were washed with high salt buffer, solubilized with the nonionic detergent Nikkol and applied to an SRP-Sepharose affinity column. The column was washed and eluted as described in Methods. Equivalent amounts of the load (*l*), flow through (*ft*), wash (*w*), and elution (*e*) fractions were separated by SDS-PAGE and immunoblotted for SR $\alpha$  and SR $\beta$  as indicated.

matography on SRP-Sepharose resins (Gilmore et al., 1982b; Gilmore and Blobel, 1983; Tajima et al., 1986). In contrast, when extracts were prepared from trypsin-digested membranes, intact SR $\beta$  was recovered in the flow through and wash fractions. The amount of SR $\beta$  recovered was roughly proportional to the amount of SR $\alpha$  that was degraded (Fig. 1 B, lanes 5–20). Thus, we conclude that SR $\alpha$  is required for binding of the SR $\alpha$ /SR $\beta$  complex to SRP. In the simplest scenario this would occur through a direct interaction of SR $\alpha$  with SRP. However, more complicated possibilities, e.g., that SR $\alpha$  is an allosteric regulator of SR $\beta$  and, as such, is required for SR $\beta$  to bind SRP, cannot be ruled out from these data.

#### Membrane Association of SR

SR $\alpha$  was proposed to associate with the ER membrane through its amino-terminal region (Hortsch et al., 1985; Lauffer et al., 1985; Andrews et al., 1989). The two stretches

of hydrophobic amino acids that are present in this region are, however, of insufficient length to function as conventional transmembrane helices. The SR $\alpha$ /ER membrane interaction may involve protein-lipid and/or protein-protein interactions, and one function of SR $\beta$  might be to tether SR $\alpha$  to the ER membrane. To determine whether the SR subunits behave as peripheral membrane proteins or as integral membrane proteins, we performed carbonate extraction of microsomal vesicles. Carbonate solutions at pH 11.2 are nonspecific protein denaturants that disrupt protein-protein interactions that bind peripheral membrane proteins to the membrane but do not disrupt protein-lipid interactions that retain integral membrane proteins in the lipid bilayer (Fujiki et al., 1982; Davis and Model, 1985). After extraction, lipid bilayers containing integral membrane proteins are collected by centrifugation leaving peripheral membrane proteins in the supernatant. When microsomal vesicles were subjected to carbonate extraction at pH of 11.2, neither SR $\alpha$  nor SR $\beta$  par-



**Figure 2.** Membrane association of SR. (A) Alkaline extraction of canine microsomal membranes. Microsomes were extracted at either pH 11.2 (lanes 1-3), 12.0 (lanes 4-6), or 13.0 (lanes 7-9) and then pelleted by centrifugation. Equivalent amounts of the total reaction mixture (*t*) and of the supernatant (*s*), and pellet (*p*) fractions were separated by SDS-PAGE and immunoblotted for SR $\alpha$ , SR $\beta$ , and mp30 as indicated. (B) Triton X-114 extraction of canine microsomal membranes. Membranes were either mock proteolyzed (lanes 1-3) or treated with 25  $\mu$ g/ml trypsin (lanes 4-6) and then extracted with the detergent Triton X-114 as described in Methods. Equivalent amounts of the total reaction mixture (*t*), and of the "detergent-poor" supernatant (*s*), and "detergent-rich" pellet (*p*) fractions were separated by SDS-PAGE and immunoblotted for SR $\alpha$ , SR $\beta$ , and mp30 as indicated.

tioned cleanly into either the supernatant or the pellet fraction (Fig. 2 A, lanes 1-3). Both subunits were partially extracted, with more SR $\alpha$  being released from the membrane than SR $\beta$ . In contrast, mp30, a control bona fide ER integral membrane protein of unknown function (Tajima et al., 1986) (J. Moskaug and P. Walter, unpublished observation), was exclusively recovered in the membrane pellet fraction. To obtain more stringent extraction conditions, we adjusted the pH to increasingly alkaline conditions. At pH 12.0 and 13.0 most of SR $\alpha$  and approximately half of SR $\beta$  was extracted, while most of the mp30 still pelleted with the membranes (Fig. 2 A, lanes 4-9). Taken together, these results suggest that SR $\beta$  has a more hydrophobic character than SR $\alpha$ , but do not allow SR $\beta$  to be unambiguously characterized as an integral membrane protein.

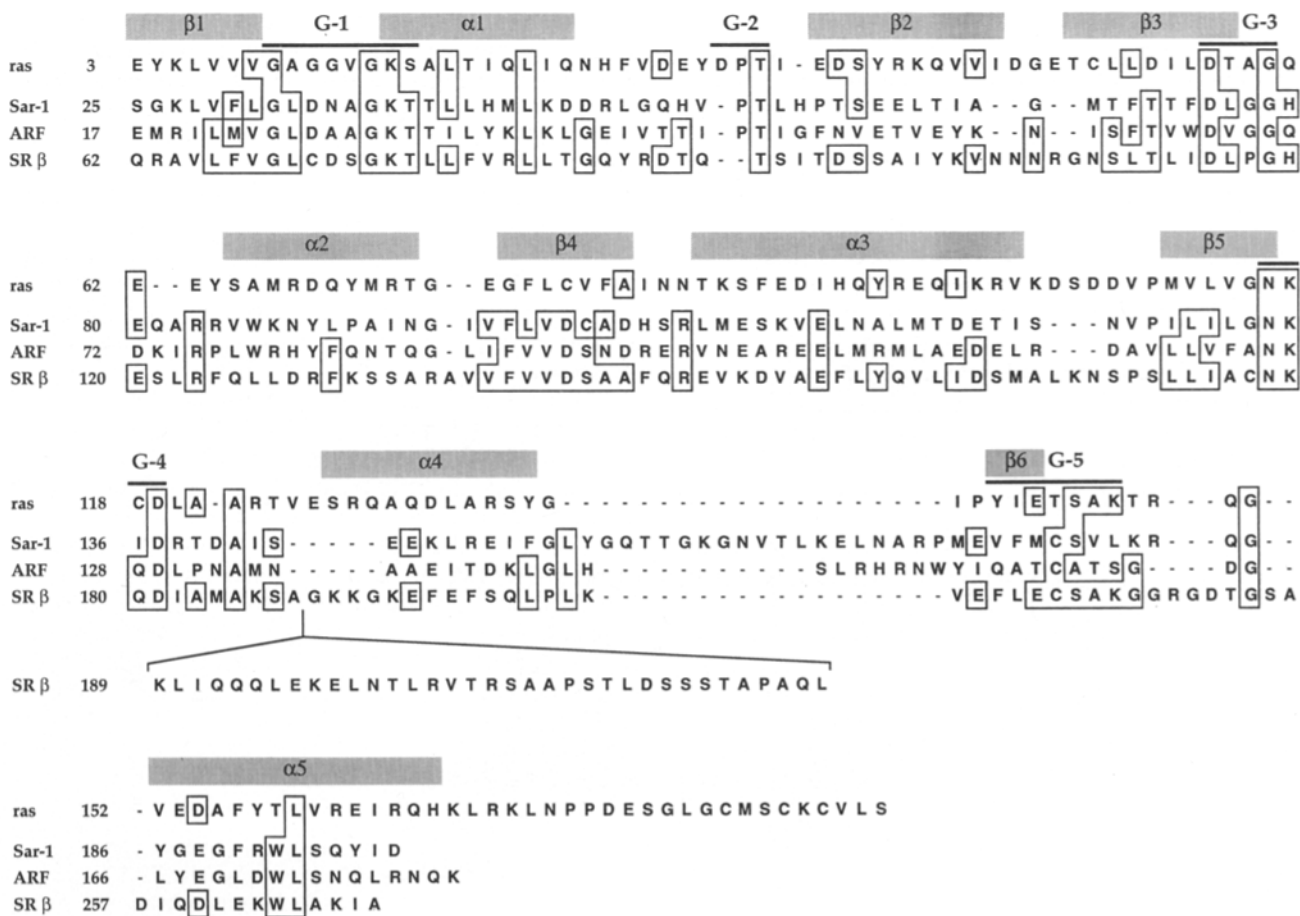
Because of this anomalous behavior, we examined the membrane association of SR by another, independent, means. Solutions of the nonionic detergent Triton X-114 undergo a phase separation when warmed from 4°C to room temperature, and proteins dissolved in the detergent solution partition between the hydrophilic, detergent-poor supernatant phase and the hydrophobic, detergent-rich pellet phase according to their hydrophobicity (Bordier, 1981). Usually, integral membrane proteins are exclusively recovered in the detergent-rich phase. The analysis of a Triton X-114 extract of microsomal vesicles is shown in Fig. 2 B. Both SR $\alpha$  and SR $\beta$  again distributed ambiguously into both detergent-poor supernatant and detergent-rich pellet fractions (lanes 1-3), while mp30 behaved as a true integral membrane protein, partitioning exclusively into the detergent-rich pellet fraction.

The ambiguous results obtained in these experiments must be caused by structural features of SR that distinguish it from "classical" integral and peripheral membrane proteins. SR $\alpha$  is a highly charged, hydrophilic molecule (Lauffer et al., 1985), and SR $\alpha$  and SR $\beta$  bind tightly to one another (Tajima et al., 1986). We therefore considered the possibility that, although SR $\beta$  may be an integral membrane protein, its hydrophobic character may be obscured during the fractionation procedures described above because of its association with the large, hydrophilic SR $\alpha$  moiety. To test this hypothesis, we again took advantage of the differential sensitivity to trypsin digestion of SR $\alpha$  and SR $\beta$  (Fig. 1 A). At 25  $\mu$ g/ml trypsin, SR $\alpha$  was almost completely degraded, while SR $\beta$  was essentially unaffected (Fig. 2 B, compare lane 4 with lane 1). Repeating the partitioning after Triton X-114 extraction of such trypsinized membranes (Fig. 2 B, lanes 4-6), resulted in the almost quantitative partitioning of SR $\beta$  into the detergent-rich phase, similar to the mp30 control. These results suggest that SR $\beta$  is indeed a true integral membrane protein and, as such, could function to anchor SR $\alpha$  to the membrane.

### Cloning of SR $\beta$

To confirm this conclusion and to address the structure/function relationship of the SR subunits in further detail, we isolated and sequenced a cDNA clone encoding SR $\beta$ . A canine cDNA clone was obtained by screening an expression library with a monoclonal antibody directed against the SR $\beta$  protein (see Materials and Methods) and its sequence predicts a protein of the correct molecular mass (~30 kD). Peptide sequence data was obtained from the amino terminus and from





**Figure 4.** SR $\beta$  secondary structure prediction and homology to Sar-1, ARF and *ras*. Three criteria were used to generate this alignment: (a) placement of GTPase motifs G-1 through G-5 as defined by Bourne et al. (Bourne et al., 1991) and indicated by the solid lines over the sequence; (b) amino acid identity between SR $\beta$  and the other proteins (boxes); and (c) identification of potential secondary structures in SR $\beta$  consistent with those known for *ras* from x-ray crystallographic studies (gray boxes over sequence:  $\alpha$  = alpha helix,  $\beta$  = beta sheet). G-1: GXXXXGK(T/S), X = any amino acid; G-2 varies between subfamilies of GTPases, but always contains a critical threonine; G-3: DXXG; G-4: NKXD; G-5, like G-2, varies between subfamilies, but is conserved within a family (see text for discussion of assignment of this motif). The bracketed sequence beginning with SR $\beta$  K189 is predicted to be an insertion that may form a loop on the surface of the core GTPase domain. All sequences displayed are mammalian: rat *ras* (Ruta et al., 1986), murine Sar-1 (Shen et al., 1993), and human ARF (Bobak et al., 1989).

acids G85 to G108) is not as readily assigned, as this part of the protein differs among different members of the GTPase superfamily. It would be premature to assign secondary structure here. The G-2 motif contains a conserved threonine residue (T35 in *ras*) which in *ras* is essential for GTP hydrolysis and hydrogen bonds to the  $\gamma$ -phosphate of the bound GTP. T92 of SR $\beta$  may correspond to this residue.

Alignment of the carboxyl-terminal 80 residues of SR $\beta$  is more problematic, as the remainder of the GTPase domain of *ras* (i.e., helices  $\alpha$ 4 and  $\alpha$ 5 and strand  $\beta$ 6) comprises only an additional 40 amino acids. We believe that two considerations resolve the difficulty here: (a) a carboxyl-terminal helix,  $\alpha$ 5, packs against the  $\beta$ -sheet, in effect closing the fold in each of the known GTPase structures; and (b) the loops between strand  $\beta$ 5 and helix  $\alpha$ 4, and those between strand  $\beta$ 6 and helix  $\alpha$ 5 have variable lengths in the different GTPase subfamilies. Secondary structure analysis and inspection of sequence conservation suggests that the carboxyl-terminal

13 amino acids of SR $\beta$  most likely form the carboxyl-terminal helix  $\alpha$ 5. This putative helix  $\alpha$ 5 in SR $\beta$  would include conserved residues (e.g., DxxxWL). Furthermore, it is preceded by a glycine-rich region consistent with the presence of a surface loop and this candidate loop region is preceded by a seven residue sequence (FLECSAK) which shows substantial similarity to the sequence of strand  $\beta$ 6 and motif G-5 in *ras* (YIETSAK).

If these assignments hold, the amino-terminal end (to approximately residue 187) and the carboxyl-terminal end (beginning approximately with residue 241) of the GTPase fold are effectively "pinned." We, therefore, propose that the remaining residues form the missing helix  $\alpha$ 4 and a large loop, possibly between strand  $\beta$ 5 and helix  $\alpha$ 4. This is indicated by the large insertion after motif G-4 in Fig. 4. The position of helix  $\alpha$ 4 remains speculative, however. Such a large surface loop could be a key element in the structure and function of this protein, possibly mediating binding to its effectors.

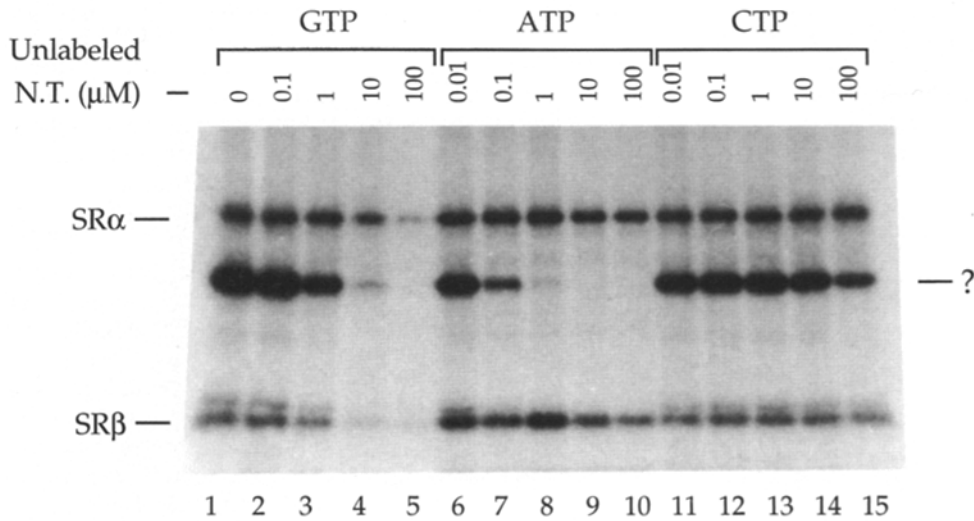


### GTP Binding of SR $\alpha$ and SR $\beta$

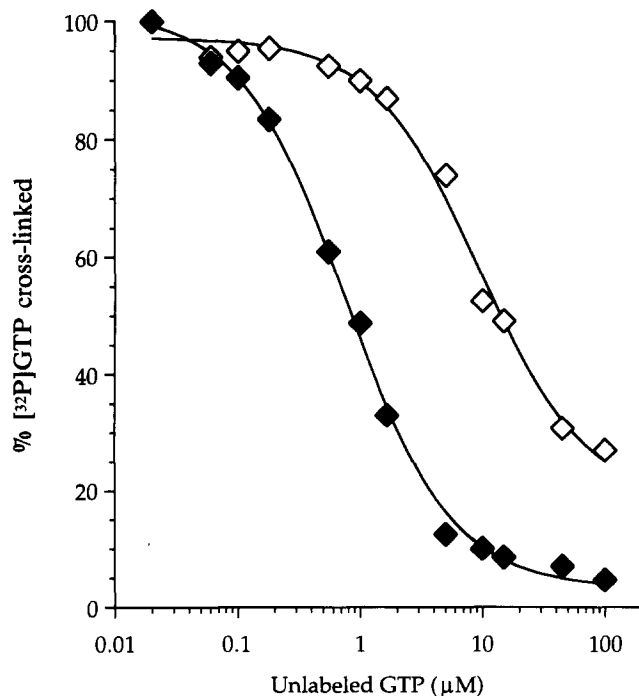
To confirm experimentally that SR $\beta$  binds GTP, direct UV cross-linking was used to create covalent nucleotide-protein adducts (Nath et al., 1985; Pashev et al., 1991; Miller et al.,

1993). When  $\alpha$ -[ $^{32}$ P] GTP is included in the reaction, GTP binding to the individual SR $\alpha$  and SR $\beta$  chains can be analyzed by autoradiography after their separation by SDS-polyacrylamide gel electrophoresis (Fig. 5 A). Using

A



B



**Figure 5.** SR $\alpha$  and SR $\beta$  specifically crosslink to GTP. (A) GTP crosslinking assay. Lane 1, purified SR was incubated with  $\alpha$ -[ $^{32}$ P]GTP and then UV irradiated to crosslink bound GTP to protein. In lanes 4–5, unlabeled GTP was included in the incubation at the indicated concentration. In lanes 6–10, unlabeled ATP was included in the incubation at the indicated concentration. In lanes 11–15, unlabeled CTP was included in the incubation at the indicated concentration. The reaction products were separated by SDS-PAGE and visualized by autoradiography. Bands corresponding to SR $\alpha$  and SR $\beta$  are indicated as is a contaminant band (labeled “?”). This contaminant is present in SRP-Sepharose-purified SR but not in immunopurified SR (not shown). (B) Quantitation of GTP crosslinking to SR. The amount of labeling of SR $\alpha$  ( $\square$ ) and SR $\beta$  ( $\blacklozenge$ ) at a given concentration of unlabeled competitor GTP was determined by densitometry and plotted against the log of the concentration of the competitor GTP.



this technique with purified SR, both SR $\alpha$  and SR $\beta$  were labeled with  $\alpha$ -[ $^{32}$ P] GTP, as was an unidentified contaminant band (Fig. 5 A, lane I). The identity of the SR $\alpha$  and SR $\beta$  bands was confirmed by immunoprecipitation (not shown). Control reactions using bovine serum albumin and lysozyme showed no labeling of these proteins (not shown), indicating that, as previously established (Miller et al., 1993), the cross-linking reaction is specific for GTP-binding proteins. To further corroborate binding specificity, we added increasing amounts of unlabeled nucleotide to the reaction (lanes 2–15). Unlabeled GTP inhibited the labeling of SR $\alpha$  and SR $\beta$  (lanes 1–5), while neither unlabeled ATP (lanes 6–10) nor unlabeled CTP (lanes 11–15) showed this effect. In contrast, labeling of the unidentified contaminant band was readily competed by ATP, suggesting that nucleotide binding to this protein is not specific for GTP. The IC $_{50}$  is the amount of unlabeled nucleotide required to inhibit the labeling of SR $\alpha$  and SR $\beta$  by 50% and it approximates the affinity of the protein for that nucleotide (Limbird, 1986). From the data shown in Fig. 5 B, the IC $_{50}$  for SR $\alpha$  is  $\sim$ 10  $\mu$ M and that for SR $\beta$  is  $\sim$ 1  $\mu$ M. Thus, we conclude that both SR $\alpha$  and SR $\beta$  bind GTP specifically albeit with relatively low affinity when compared to other GTPases such as *ras*.

## Discussion

We have shown that SR $\beta$  is a new member of the GTPase superfamily and have experimentally confirmed the ability of SR $\beta$  to bind GTP specifically. To our knowledge, it is the first such protein that is also a bona fide integral membrane protein. One other transmembrane protein, GP85, has been shown to bind GTP (Lokeshwar and Bourguignon, 1992). GP85, however, is a radically different type of GTP-binding protein that does not contain the consensus motifs common to the GTPase super family typified by *ras* (Bourne et al., 1991). We have identified a gene encoding a closely related yeast SR $\beta$  homologue that shares the features discussed for mammalian SR $\beta$ . Preliminary experiments from our laboratory indicate that disruption of the yeast SR $\beta$  gene leads to an identical phenotype to that of cells that have been deleted for the genes encoding SR $\alpha$  or any of the SRP components (S. Ogg and P. Walter, unpublished observation). This lends additional support to the assignment of the predicted yeast protein as an SRP receptor subunit, and provides in vivo evidence for the importance of SR $\beta$  in the SRP-dependent protein targeting reaction. Furthermore, we have demonstrated that SR $\alpha$  is a peripheral membrane protein that is required for the interaction of SRP with SR, while SR $\beta$  binds tightly to SR $\alpha$  and is predicted to span the ER membrane. These data suggest a model in which SR $\alpha$  mediates SRP binding (and thus, may regulate the GTPase cycle of SRP-54), while SR $\beta$  tethers SR $\alpha$  to the membrane. The unexpected discovery of the guanine nucleotide-binding domain in SR $\beta$ , however, makes it likely that its role is more complex than that of a passive membrane anchor.

GTP binding and hydrolysis by SRP54 is regulated by its interactions with different components of the targeting machinery (Miller et al., 1993). It is likely that the guanine nucleotide occupancy of SR $\alpha$  is also regulated, possibly by interaction with SRP, SR $\beta$ , or the translocon components. As SRP forms a functional targeting complex with the ribosome and nascent chains, so may SR form a complex with

translocon components rendering them capable of accepting the targeting complex. Thus, the GTPases in SR and SRP would function as “molecular match makers” that establish the ribosome/translocon junction. Cycles of GTP binding and hydrolysis may guide the components through the sequential steps of a complex reaction.

The physiological importance of guanine nucleotide binding to SR $\beta$  still remains to be demonstrated. As the GTPase domain of SR $\beta$  is evolutionary highly conserved, however, we consider it very likely that nucleotide binding to SR $\beta$  is of functional significance. We can envision two different roles that a GTPase switch in SR $\beta$  could play. First, as for SRP54 and SR $\alpha$ , a cycle of GTP binding and hydrolysis on SR $\beta$  may be required for protein targeting and the initiation of translocation. This cycle might be regulated by specific effectors, such as SR $\alpha$  or translocon components, which could serve to enhance the fidelity of targeting and/or the assembly of the ribosome/translocon junction, to assure tight coupling of targeting to assembly of the translocon, or to regulate the activity of the translocon in response to the secretory needs of the cell.

According to a second, conceptually distinct hypothesis, the information flow would be reversed. Thus, the GTPase switch in SR $\beta$  may not be instrumental for targeting and translocation per se, but rather be set by these events. According to this scenario, effectors of SR $\beta$  would be used to adapt other cellular processes to the activity of the translocon. Such events could be downstream of protein translocation assuring that the secretory pathway has sufficient capacity to handle the load of proteins entering the ER. Alternatively, effectors could feed back on the synthesis of signal sequence-bearing proteins, thereby assuring that such proteins are only made if sufficient translocation sites are available to accommodate them.

The challenge now is to decipher the individual roles of the three directly interacting GTPases—SRP54, SR $\alpha$  and SR $\beta$ —that participate in protein targeting. Although still rather complex, the combination of the available biochemical and genetic tools should render this goal experimentally accessible.

We thank Jim Kielly, Chris Carrera, and Joachim Herz for their valuable technical advice, and Doug Freymann, Saira Mian, Alfonso Valencia, and Mark Boguski for their helpful suggestions regarding the sequence analyses. We also thank Doug Freymann for his invaluable help in evaluating the structure predictions. The MDCK cell library was a generous gift of Dr. Siegfried Prehn.

This work was supported by grants from the National Institutes of Health and the Human Frontiers Science Foundation to P. Walter.

Received for publication 13 September 1994 and in revised form 25 October 1994.

## References

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
- Andrews, D. W., L. Lauffer, P. Walter, and V. R. Lingappa. 1989. Evidence for a two-step mechanism involved in assembly of functional signal recognition particle receptor. *J. Cell Biol.* 108:797–810.
- Bernstein, H. D., M. A. Portiz, K. Strub, P. J. Hoben, S. Brenner, and P. Walter. 1989. Model for signal sequence recognition from amino-acid sequence of 54k subunit of signal recognition particle. *Nature (Lond.)* 340: 482–486.
- Bobak, D. A., M. S. Nightingale, J. J. Murtagh, S. R. Price, J. Moss, and M. Vaughan. 1989. Molecular cloning, characterization, and expression of human ADP-ribosylation factors: two guanine nucleotide-dependent activators of cholera toxin. *Proc. Natl. Acad. Sci. USA.* 86:6101–6105.

- Bordier, C. 1981. Phase separation of integral membrane proteins in Triton X-114. *J. Biol. Chem.* 256:1604-1607.
- Bourne, H. R., D. A. Sanders, and F. McCormick. 1990. The GTPase superfamily: a conserved switch for diverse cell functions. *Nature (Lond.)* 348:125-132.
- Bourne, H. R., D. A. Sanders, and F. McCormick. 1991. The GTPase superfamily: conserved structure and molecular mechanism. *Nature (Lond.)* 349:117-127.
- Brauer, A. W., C. L. Oman, and M. N. Margolies. 1984. Use of *o*-Phthalaldehyde to reduce background during automated Edman degradation. *Anal. Biochem.* 137:134-142.
- Brown, J. D., B. C. Hann, K. F. Medzihradzky, M. Niwa, A. L. Burlingame, and P. Walter. 1994. Subunits of the *Saccharomyces cerevisiae* signal recognition particle required for its functional expression. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:4390-4400.
- Connolly, T., and R. Gilmore. 1986. Formation of a functional ribosome-membrane junction during translocation requires the participation of a GTP-binding protein. *J. Cell Biol.* 103:2253-2261.
- Connolly, T., and R. Gilmore. 1989. The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. *Cell.* 57:599-610.
- Crowley, K. S., S. Liao, V. E. Worrell, and G. D. Reinhart, and A. E. Johnson. 1994. Secretory proteins move through the ER membrane via an aqueous, gated pore. *Cell.* 78:461-471.
- Davis N. G., and P. Model. 1985. An artificial anchor domain: hydrophobicity suffices to stop transfer. *Cell.* 41:607-614.
- Fujiki, Y., A. L. Hubbard, S. Fowler, and P. B. Lazarow. 1982. Isolation of intracellular membranes by means of sodium carbonate treatment: application to endoplasmic reticulum. *J. Cell Biol.* 93:97-102.
- Gilmore, R., and G. Blobel. 1983. Transient involvement of signal recognition particle and its receptor in the microsomal membrane prior to protein translocation. *Cell.* 35:677-685.
- Gilmore, R., G. Blobel, and P. Walter. 1982a. Protein translocation across the endoplasmic reticulum. I. Detection in the microsomal membrane of a receptor for the signal recognition particle. *J. Cell Biol.* 95:463-469.
- Gilmore, R., P. Walter, and G. Blobel. 1982b. Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. *J. Cell Biol.* 95:470-477.
- Görlich, D., and T. A. Rapoport. 1993. Protein translocation into proteoliposomes reconstituted from purified components of the endoplasmic reticulum membrane. *Cell.* 75:615-630.
- Hortsch, M., D. Avossa, and D. I. Meyer. 1985. A structural and functional analysis of the docking protein. *J. Biol. Chem.* 260:9137-9145.
- Jurnak, F. 1985. Structure of the GDP domain of EF-Tu and location of the amino acids homologous to *ras* oncogene proteins. *Science (Wash. DC)* 230:32-36.
- Krieg, U. C., P. Walter, and A. E. Johnson. 1986. Photocrosslinking of the signal sequence of nascent preprolactin to the 54-kilodalton polypeptide of the signal recognition particle. *Proc. Natl. Acad. Sci. USA.* 83:8604-8608.
- Kurzhalia, T. V., M. Wiedemann, A. S. Girshovich, E. S. Bochkareva, H. Bielka, and T. A. Rapoport. 1986. The signal sequence of nascent preprolactin interacts with the 54K polypeptide of the signal recognition particle. *Nature (Lond.)* 320:634-636.
- Lambright, D. G., J. P. Noel, H. E. Hamm, and P. B. Sigler. 1994. Structural determinants for activation of the alpha-subunit of a heterotrimeric G protein. *Nature (Lond.)* 369:621-628.
- Lauffer, L., P. D. Garcia, R. N. Harkins, L. Coussens, A. Ullrich, and P. Walter. 1985. Topology of the SRP receptor in the endoplasmic reticulum membrane. *Nature (Lond.)* 318:334-338.
- Limbird, L. E. 1986. *Cell Surface Receptors: A Short Course on Theory and Methods.* Martinus Nijhoff Publishing. Boston.
- Lokeshwar, V. B., and L. Y. W. Bourguignon. 1992. The lymphoma transmembrane glycoprotein GP85 (CD44) is a novel guanine nucleotide-binding protein which regulates GP85 (CD44)-ankyrin interaction. *J. Biol. Chem.* 267:22073-22078.
- Maniatis, T., E. F. Fritsch and J. Sambrook. 1982. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.
- Meyer, D. I., D. Louvard, and B. Dobberstein. 1982. Characterization of molecules involved in protein translocation using a specific antibody. *J. Cell Biol.* 92:579-583.
- Miller, J. D., H. Wilhelm, L. Gierasch, R. Gilmore, and P. Walter. 1993. GTP binding and hydrolysis by the signal recognition particle during initiation of protein translocation. *Nature (Lond.)* 366:351-354.
- Nath, J. P., G. R. Eagle, and R. H. Himes. 1985. Direct photoaffinity labeling of tubulin with guanosine 5'-triphosphate. *Biochemistry.* 24:1555-1560.
- Noel, J. P., H. E. Hamm, and P. B. Sigler. 1993. The 2.2 Å crystal structure of transducin-alpha complexed with GTP gamma S. *Nature (Lond.)* 366:654-663.
- Pai, E. F., W. Kabsch, U. Krengel, K. C. Holmes, J. John, and A. Wittinghofer. 1989. Structure of the guanine-nucleotide-binding domain of the *H-ras* oncogene product p21 in the triphosphate conformation. *Nature (Lond.)* 341:209-214.
- Pai, E. F., U. Krengel, G. A. Petsko, R. S. Goody, W. Kabsch, and A. Wittinghofer. 1990. Refined crystal structure of the triphosphate conformation of H-ras p21 at 1.35 Å resolution: implications for the mechanism of GTP hydrolysis. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2351-2359.
- Pashev, I. G., S. I. Dimitrov, and D. Angelov. 1991. Crosslinking proteins to nucleic acids by ultraviolet laser irradiation. *Trends Biochem. Sci.* 16:323-326.
- Rapiejko, P. J., and R. Gilmore. 1992. Protein translocation across the endoplasmic reticulum requires a functional GTP binding site in the alpha-subunit of the signal recognition particle receptor. *J. Cell Biol.* 117:493-503.
- Rawlings, N., K. Ashman, and B. Wittmann-Liebold. 1983. Computerised version of the Chou and Fasman protein secondary structure predictive method. *Internat. J. Pept. Protein Res.* 22:515-524.
- Römisch, K., J. Webb, J. Herz, S. Prehn, R. Frank, M. Vingron, and B. Dobberstein. 1989. Homology of the 54K Protein of signal recognition particle, docking protein, and two *E. coli* proteins with putative GTP-binding domains. *Nature (Lond.)* 340:478-482.
- Ruta, M., R. Wolford, R. Dhar, D. Defeo-Jones, R. W. Ellis, and E. M. Scholnick. 1986. Nucleotide sequence of the two rat cellular *rasH* genes. *Mol. Cell. Biol.* 6:1706-1710.
- Shen, K. A., C. M. Hammond, and H. P. Moore. 1993. Molecular analysis of SARI-related cDNAs from a mouse pituitary cell line. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 335:380-385.
- Simon, S. M., and G. Blobel. 1992. Signal peptides open protein-conducting channels in *E. coli*. *Cell.* 69:677-684.
- Stanley, K. K. 1983. Solubilization and immune-detection of beta-galactosidase hybrid proteins carrying foreign antigenic determinants. *Nucleic Acids Res.* 11:4077-4092.
- Stanley, K. K., and J. P. Luzio. 1984. Construction of a new family of high efficiency bacterial expression vectors: identification of cDNA clones coding for human liver proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1429-1434.
- Tajima, S., L. Lauffer, V. L. Rath, and P. Walter. 1986. The signal recognition particle is a complex that contains two distinct polypeptide chains. *J. Cell Biol.* 103:1167-1178.
- Walter, P., and G. Blobel. 1980. Purification of membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. *Proc. Natl. Acad. Sci. USA.* 77:7112-7116.
- Walter, P., and G. Blobel. 1982. Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. *Nature (Lond.)* 299:691-698.
- Walter, P., and G. Blobel. 1983a. Preparation of microsomal membranes for cotranslational protein translocation. *Methods Enzymol.* 96:84-93.
- Walter, P., and G. Blobel. 1983b. Signal recognition particle: a ribonucleoprotein required for cotranslational translocation of proteins, isolation and properties. *Methods Enzymol.* 96:682-691.
- Walter, P., and A. E. Johnson. 1994. Signal sequence recognition and protein targeting to the endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.* 10:87-119.