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Each of the Activities of Signal Recognition Particle (SRP) Is Contained within a Distinct Domain: Analysis of Biochemical Mutants of SRP

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Summary

Signal recognition particle (SRP), a small ribonucleoprotein required for targeting secretory proteins to the ER, has three known functions: signal recognition, elongation arrest, and translocation promotion. Because SRP is inactivated by the sulfhydryl alkylating reagent N-ethylmaleimide (NEM), we have attempted to establish structure-function relationships within SRP by assembling particles in which a single protein is modified. Alkylation of the 68/72 kd protein of SRP yields a particle that arrests elongation but fails to promote translocation and no longer interacts with SRP receptor. Alkylation of the 54 kd protein yields a particle that fails to recognize signal sequences. This approach has allowed us to map activities to specific protein domains on SRP, and should be generally useful for analyzing other ribonucleoproteins.

Introduction

The role of SRP in the targeting of nascent secretory proteins to the endoplasmic reticulum has been reviewed (Walter et al., 1984). Three distinct activities of the particle can be assayed in the wheat germ cell-free translation system. The first activity is signal recognition, by which SRP binds to ribosomes that are synthesizing secretory proteins with high affinity ($\sim 10^{-9}$ M; Walter et al., 1981), and which can be measured in cosedimentation experiments. Concomitant with signal recognition, SRP specifically blocks further elongation of the presecretory nascent chain (Walter and Blobel, 1981). This elongation arrest activity can be assayed in vitro as an inhibition of full-length presecretory chain synthesis, and as the appearance of an arrested fragment in synchronized translation systems. Finally, SRP promotes translocation across the endoplasmic reticulum membrane (Walter and Blobel, 1980) by its interaction with the SRP receptor, a resident protein of the ER membrane; this activity can be measured in a number of ways, such as the removal of the signal sequence from presecretory proteins by signal peptidase, a lumenal ER protein (signal cleavage), or cosedimentation of nascent or full-length secretory proteins with ER membranes, or protection of these proteins from exogenously added proteases.

SRP is comprised of a number of different components. It contains four different proteins (two monomers composed of a 19 kd and a 54 kd polypeptide, and two heterodimers, one composed of a 9 kd and a 14 kd polypeptide and the other composed of a 68 kd and a 72 kd polypeptide, respectively) and one 300 nucleotide molecule of RNA (7SL RNA, here referred to as SRP RNA) (Walter and Blobel, 1983a, Siegel and Walter, 1985).

Approximately 100 nucleotides at the 5′ end and 45 nucleotides at the 3′ end of SRP RNA are homologous to the human Alu right monomer sequence (Ullu et al., 1982). The central "S" segment of 155 nucleotides shows no homology to Alu DNA.

It has been our goal to understand the structural and functional organization of SRP, i.e., the relationship between its multiple components and its multiple activities. Our approach has been to create biochemically the equivalent of mutations in the particle; our hope was to create particles that were lacking a single activity, and in so doing to assign functional domains on the particle, and to address questions concerning the dependency relations of the various SRP activities that could be assayed in vitro.

We have described previously two such "mutations." In one set of experiments (Siegel and Walter, 1985), protein and RNA components were fractionated into five homogeneous subfractions. Because fully active SRP was obtained upon recombining these fractions, we tried to determine what would happen if one of the five fractions was left out of the reconstitution protocol. We found that one such particle, SRP(−9/14), was active in promoting translocation even though it had completely lost its elongation arrest activity. From these experiments it was determined that elongation arrest was not a prerequisite for protein translocation and that the SRP receptor was required for protein translocation even in the absence of elongation arrest.

In a second set of experiments (Siegel and Walter, 1986), micrococcal nuclease was used to generate a subparticle, which was missing, in addition to the 9/14 kd protein, essentially all of the Alu-like sequences of the RNA. This subparticle, SRP(S), was stable to ion-exchange chromatography and sucrose gradient sedimentation and thus could be thought of as an independent structural domain in the particle. SRP(S) had the same activity as SRP(−9/14), namely, it was active in signal recognition and translocation promotion but inactive in elongation arrest. Thus we established that SRP can be divided into two domains: an "Alu domain", which contains the elongation arrest activity of SRP, and an "S domain", which contains its signal recognition and protein translocation functions.

In further dissecting the latter two functions to specific domains within the S region, these two approaches failed. It should be noted, however, that single omission and nuclease digestion represent rather severe structural alterations of the particle, and therefore it is not surprising that in most cases the particle is completely inactivated. For this reason, we sought a way to introduce more subtle domain specific lesions into SRP.

For this approach, we chose the sulfhydryl specific alkylating agent N-ethylmaleimide (NEM). SRP is known to be exquisitely sensitive to this agent (Walter and Blobel, 1980), losing even its ribosome binding activity (Walter et

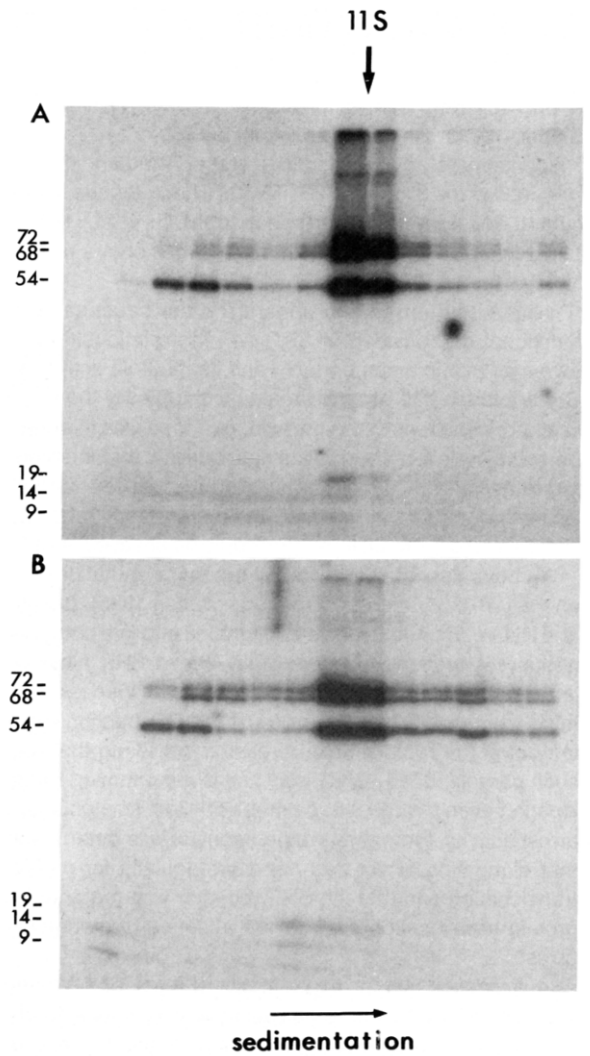

Figure 1. Sucrose Gradient Analysis of Unmodified and NEM-Treated SRP

Fifty microliters of ^{125}I-SRP (~150 fmol) was treated with 1 mM NEM for 30 min at 25°C prior to addition of DTT to 10 mM. In the unmodified sample, DTT was added prior to the addition of NEM. Samples were loaded onto 13 ml 5%–20% sucrose gradients, spun for 20 hr at 40,000 rpm in an SW40 rotor in the Beckman L8 ultracentrifuge, and fractionated into 13 1 ml fractions. Samples were resolved by SDS-PAGE and visualized by autoradiography with an intensifying screen. Exposure time was 7 days. (A) is unmodified and (B) modified SRP. The left-hand side represents the top of the gradient. The molecular weights of the SRP polypeptides are indicated alongside the autoradiograms. The 11S peak of SRP is indicated.

al., 1981). Furthermore, when ^{3}H-NEM was used to label SRP, several of the polypeptides were labeled (Walter and Blobel, 1980). We hoped that if we could modify specific proteins in SRP with NEM, then we would inactivate only the functions encoded by the modified protein.

Results

NEM Does Not Cause the Disassembly of SRP

One explanation for the complete inactivation of SRP by NEM is that NEM causes SRP to disassemble. We tested

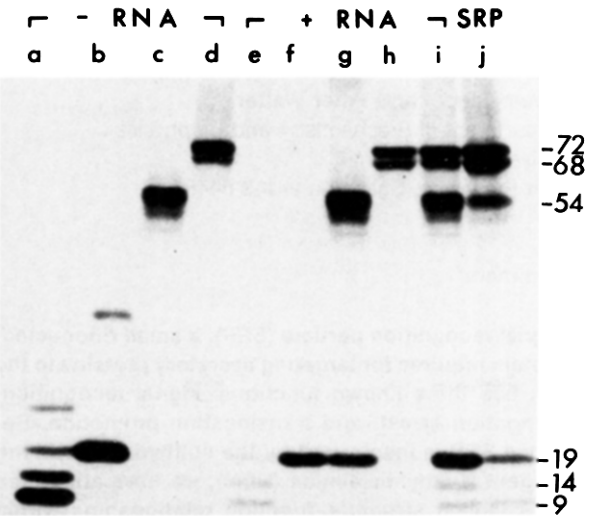

Figure 2. ^{3}H-NEM Labeling of SRP Proteins and Reconstitutes

Five picomoles of each SRP protein in the absence (lanes a–d) or presence (lanes e–i) of a 10-fold molar excess of RNA was incubated with 60 μCi ^{3}H-NEM for 30 min at 25°C prior to TCA precipitation and SDS-PAGE analysis. Lanes a, e: 9/14 kd; lanes b, f: 19 kd; lanes c, g: 54 kd; and lanes d, h: 68/72 kd proteins. Lane i is a total reconstitute and lane j gradient purified SRP. The molecular weights of the SRP polypeptides are indicated.

this possibility by modifying ^{125}I-SRP with NEM and then analyzing the integrity of the particle by sucrose gradient sedimentation. In Figure 1 we show a sucrose gradient profile of unmodified (A) and modified (B) SRP. We found that a majority of the labeled protein sediments as a single peak at 11S, and thus represents intact SRP, and that therefore the inactivation of the particle by NEM must result from something other than its disassembly.

We did note that a small fraction of the labeled protein sedimented less far into the gradient. In the case of the 54 kd protein, this was true whether or not the particle was modified with NEM and probably represents an alteration of the 54 kd protein by the labeling procedure. A small effect on the binding of the 9/14 kd protein (compare the levels of 9/14 kd in the top fraction of the two gradients) to the particle was seen upon modification. In addition, the increased level of the small polypeptides in fraction 6 in the NEM-treated sample may reflect a small amount of SRP(–68/72) due to disassembly of those polypeptides.

Several SRP Proteins Are Potential Targets for NEM Inactivation

In order to determine which SRP proteins could be modified with the sulfhydryl reactive agent N-ethylmaleimide (NEM) and thus be potential targets for NEM inactivation experiments, individual SRP proteins were labeled with ^{3}H-NEM either alone or after being bound to SRP RNA under standard reconstitution conditions. The result of such a labeling experiment is shown in Figure 2. As can be seen in lanes a–d, when the proteins are free in solution, all of the SRP polypeptides can be labeled (when comparing the intensities of labeling, note that the 9 kd

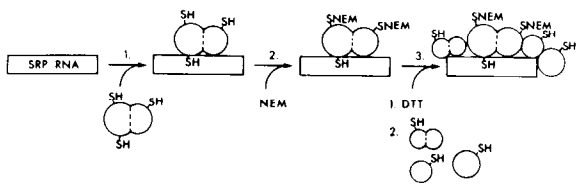

Figure 3. Generation of Singly Modified SRPs
The scheme for protecting RNA binding domains from inactivation by NEM is shown here. See Results for discussion.

protein contains two cysteine residues, as determined from the primary sequence of a cDNA clone, K. Strub and P. Walter, unpublished data). However, when the RNA was bound to the proteins in a reconstitution reaction prior to labeling, protection of the proteins to NEM modification was seen. Most strikingly, the 9 kd polypeptide was almost completely protected from modification by the RNA (compare Figure 2, lanes a and e). In addition, the labeling of most of the other proteins by ^{3}H-NEM was diminished in the presence of the RNA. One explanation for the diminution of protein labeling in the presence of the RNA is that the modification sites lie within regions of the protein involved in RNA binding. If such an explanation is correct, modification of these proteins in isolation may influence their ability to subsequently bind to the RNA in a reconstitution reaction.

With this in mind, we designed a reconstitution scheme to generate complete SRP particles in which a single protein contains the NEM modification, and this scheme is depicted in Figure 3. The salient feature of this method is that the protein is modified in the presence of the SRP RNA under conditions of RNA excess. The protein is thus driven into the bound state, and any regions involved in RNA binding are protected. First, the protein to be modified was incubated with SRP RNA under standard reconstitution conditions (Figure 3, step 1). Next, NEM was added and allowed to react for 30 min at 25°C (Figure 3, step 2), after which time DTT or glutathione was added to inactivate any residual NEM (Figure 3, step 3.1). Finally, the other SRP proteins were included in the mixture (Figure 3, step 3.2), and the particle was subjected to another round of reconstitution, thus generating complete particles with single protein components modified.

By several criteria we have demonstrated that this reconstitution scheme promotes complete assembly of particles containing NEM-modified subunits. For example, particles modified on the 19 kd and 54 kd polypeptides sedimented as completely assembled particles on sucrose gradients (data not shown). Evidence for complete assembly of particles containing modified 68/72 kd proteins is discussed below.

Phenotypes of the NEM-Modified Particles

We assayed the activity of particles generated, and compared it to ones in which the isolated protein was modified with NEM prior to reconstitution with the RNA. Using the well-established in vitro translation/translocation reactions, we determined the ability of these particles to inhibit synthesis of the preprolactin nascent chain (elongation arrest) and to promote the translocation and subsequent signal cleavage of preprolactin to prolactin (translocation promotion). The results are summarized in Table 1.

Table 1. Phenotype of NEM-Modified SRPs

Protein Modified	RNA Present during Modification?	Elongation Arrest	Protein Translocation
54	no	–	–
19 and 54	yes	–	–
19	no	+	+
19	yes	+	+
*9/14	no	–	+
*9/14	yes	+	+
*68/72	no	–	–
*68/72	yes	+	–

Proteins were modified with NEM either as isolated proteins or after reconstitution onto SRP RNA as depicted in Figure 3. Complete reconstitutes containing these modified proteins were then generated, and the activity of these reconstitutes in elongation arrest and translocation assays is shown here. * denotes particles in which the activity differs depending on whether the modification was performed on proteins in isolation or on partial reconstitutes.

We found that SRP was exquisitely sensitive to NEM modification of the 54K protein. The particle that contained such a modification, which we call SRP(54^{N}), had lost both elongation arrest and translocation functions (experiments addressing the reason for this inactivation are presented below). In contrast, SRP could tolerate a modification of the 19K protein, and retained full activity. In two cases (9/14 and 68/72), we found that the activity of the particle differed depending on whether the protein was modified or the single reconstitute was modified. In both these cases the activity of a reassembled mixture in which the protein alone was modified was equivalent to that of a reconstitute lacking that protein (loss of elongation arrest for the 9/14 and loss of both activities for the 68/72). This result is consistent with the idea that modification of these proteins affected their binding. When the 9/14 kd protein was modified in the presence of the RNA, the particle retained full activity; this is consistent with the result in Figure 2 that the labeling of these polypeptides with ^{3}H-NEM is almost completely abolished in the presence of the RNA.

A rather intriguing phenotype was found when the 68/72 kd protein was modified as a single reconstitute: the particle, which we called SRP(68/72^{N}), was able to arrest preprolactin synthesis, but could not promote its translocation across the microsomal membrane. We wished to determine the "primary defect" resulting in this phenotype, and so we studied its activity in greater detail.

Microsomal Membranes Fail to Release the Arrest Induced by SRP(68/72^{N})

Varying amounts of SRP and SRP(68/72^{N}) were added to elongation arrest and translocation assays, and the results are depicted in Figure 4. In agreement with Table 1, we found that SRP(68/72^{N}) was fully active in elongation arrest (Figure 4A, filled triangles), indicating that the

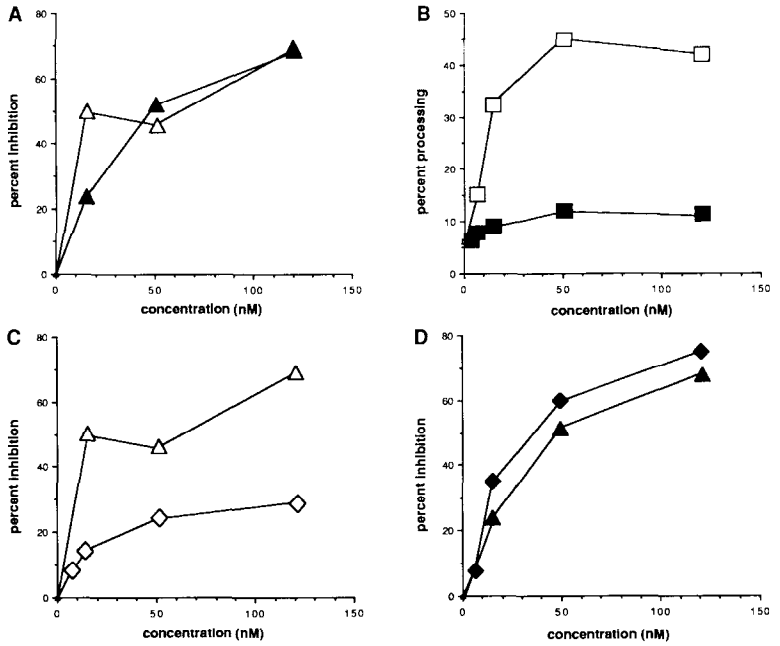

Figure 4. Activity Assay of SRP and SRP(68/72^{N})

SRP and SRP(68/72^{N}) were assembled to a final concentration of 500 nM p68/72. Varying amounts of these particles were added to elongation arrest and translocation assays, and the results quantitated by densitometry, and percent inhibition and percent translocation calculated as described (see Experimental Procedures).

(A) Percent inhibition as a function of SRP (open triangles) and SRP(68/72^{N}) (filled triangles) concentration.

(B) Percent translocation as a function of SRP (open squares) and SRP(68/72^{N}) (filled squares) concentration.

(C) Percent inhibition as a function of SRP concentration in the absence (open triangles) and the presence (open diamonds) of microsomal membranes.

(D) Percent inhibition as a function of SRP(68/72^{N}) concentration in the absence (filled triangles) and the presence (filled diamonds) of microsomal membranes.

signal recognition and elongation arrest domains were not affected by the modification. Furthermore, the NEM-modified SRP must have remained fully assembled. Any disassembly induced by NEM modification on the 68/72 kd protein would have been detected as a decrease in elongation arrest activity, since a particle lacking the 68/72 kd protein is inactive in elongation arrest (Siegel and Walter, 1985).

The amount of arrest shown here reached a maximum of about 70%. In other reconstitution reactions, we have been able to improve the arrest demonstrated by SRP (68/72^{N}) and its control reconstitute by increasing the amount of 9/14 kd protein in the reconstitution. In some cases we found that the modified particle was somewhat less active in elongation arrest activity than the control reconstitute, which may be the result of some of the protein being free in solution during the modification reaction. In contrast to its ability to inhibit the elongation of preprolactin, SRP(68/72^{N}) was completely unable to promote the translocation of preprolactin across the microsomal membrane (Figure 4B, filled squares).

Another measure of the functional interaction of SRP-arrested nascent complexes with SRP receptor in the microsomal membrane is the release of the SRP-induced arrest (Walter and Blobel, 1981). When membranes were included in translation reactions containing SRP or SRP (68/72^{N}), we found that the inhibition of synthesis induced by the control reconstitute was reduced ~3-fold (compare open triangles and open diamonds in Figure 4C), indicating that the membranes were able to release the arrest induced by SRP. Such an arrest releasing activity was *not* seen when SRP(68/72^{N}) was included in the reaction (compare filled diamonds and triangles in Figure 4D). In other words, in contrast to the case for SRP, which upon interaction with SRP receptor is released from the ribosome/nascent chain complex and free to recycle (Gilmore and Blobel, 1983), SRP(68/72^{N}) remains bound to the complex even in the presence of microsomal membranes, and the nascent chain is retained in an arrested state.

The Failure of Microsomal Membranes to Release Arrest Does Not Explain the Translocation Defective Phenotype of SRP(68/72^{N})

We wondered whether the translocation defective phenotype of SRP(68/72^{N}) was the result of the failure of SRP receptor to release the arrest induced by this particle. If such a hypothesis is correct, we would expect translocation promoting activity to be restored by inactivating the

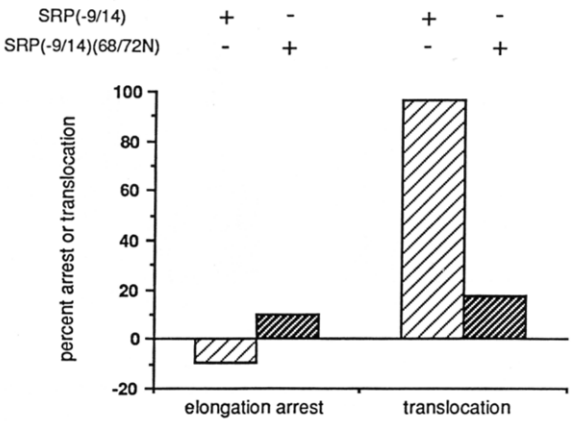

Figure 5. Arrest and Translocation Assays for SRP(−9/14), and SRP(−9/14)(68/72^N)

p68/72 was reconstituted onto SRP RNA prior to NEM modification as described. When the additional protein components were added in the final reconstitution step, p9/14 was replaced with compensating buffer to generate SRP(−9/14)(68/72^N) and its control particle. Assays were performed at 120 nM of each reconstitute. Note that neither particle significantly affects elongation; the measured values reflect the intrinsic variability of the assay.

elongation arrest function. We did this by omitting the 9/14 kd protein from the reconstitution reaction (see Siegel and Walter, 1985).

When we included SRP(−9/14)(68/72^N) (i.e., SRP lacking the 9/14 kd protein and NEM modified on the 68/72 kd protein) in a translocation assay (Figure 5), we found that, as expected, the elongation arrest was relieved by removal of the 9/14 kd protein. However, even in the absence of elongation arrest, the particle was unable to promote translocation. It seemed rather, as was the case in translation reactions containing SRP(68/72^N), that the pattern of chains synthesized in the presence of microsomal membranes differed in no way from those synthesized in their absence. We wondered whether these nascent chains were in fact ever targeted to the membrane.

SRP(68/72^N) Fails to Target Nascent Preprolactin to the Microsomal Membrane

Simple assays for the targeting of preprolactin to microsomal membranes have recently been developed. In brief, it has recently been shown that SRP will mediate the targeting only of chains associated with the ribosome (Mueckler and Lodish, 1986; Perara et al., 1986; Garcia and Walter, unpublished data; Siegel and Walter, unpublished data). The assays take advantage of this fact by enriching for ribosome-associated chains with the positively charged detergent cetyltrimethylammoniumbromide (CTABr), which precipitates the nascent peptide by virtue of its linkage to tRNA (Hobden and Cundliffe, 1978). By looking only at "nascent" chains, it has been possible to synthesize chains either in the presence or absence of SRP (the former yielding the characteristic "arrested fragment", and the latter yielding a distribution of chains of various lengths), and then to target these chains to microsomal membranes in an SRP-dependent manner.

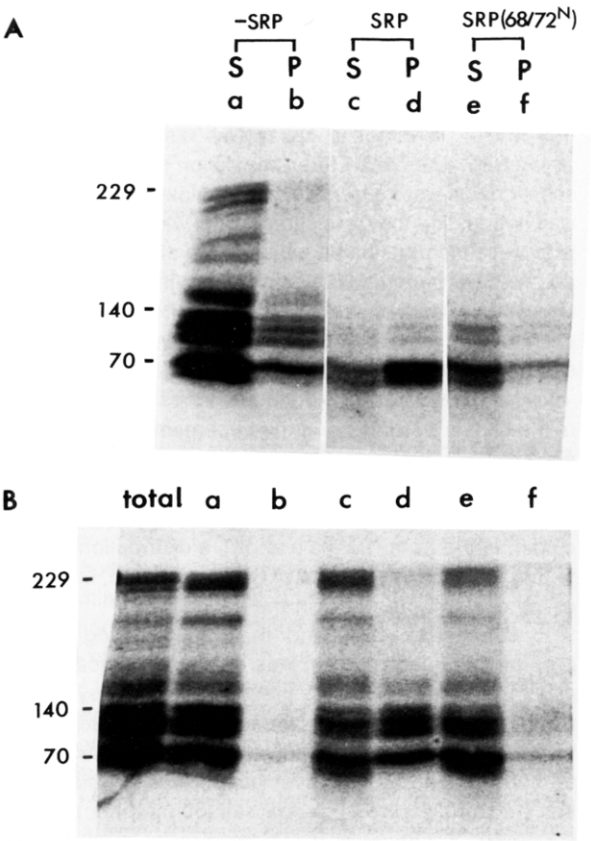

Figure 6. Targeting of Preprolactin to Microsomal Membranes

(A) No SRP was added, or SRP or SRP(68/72^N) was added to 120 nM prior to the addition of mRNA to initiate protein synthesis. After a 20 min synthesis, cycloheximide was added, and then microsomal membranes were added and incubated for 5 min at 22°C. Samples were spun for 3 min at 20 psi in the Beckman airfuge A-100/30 rotor over a 50 μl sucrose cushion. Supernatants and pellets were analyzed by CTABr precipitation.

(B) After synthesis of preprolactin for 14 min at 22°C, cycloheximide was added. Then SRP was added to 80 nM and incubated for 10 min, followed by incubation with microsomal membranes for 5 min and subsequent CTABr precipitation as in (A).

Lanes a, c, e: supernatant; lanes b, d, f: pellet. Lanes a, b, −SRP; lanes c, d, +SRP; lanes e, f, +SRP(68/72^N). Positions of preprolactin (229 amino acids), a 140 amino acid length chain, and the "arrested fragment" (70 amino acids) are indicated.

In Figure 6A, translation extracts were programmed with synthetic preprolactin mRNA in the absence or presence of SRP and allowed to synthesize chains for about 20 min. Cycloheximide was added to inhibit further elongation followed by the addition of microsomal membranes. Cytoplasmic and membrane fractions were separated by sedimentation and each was analyzed by CTABr precipitation as described in Experimental Procedures. Note that in the absence of SRP (lanes a and b), chains ranging from about 50 amino acids in length to chains approximately full length are detected. However, when SRP or SRP(68/72^N) is added to the translation (lanes c–f), only the shorter chains are seen, indicating that both SRP and SRP(68/72^N) inhibit elongation.

Due to this inhibition of translation, different amounts of translation products were obtained in the absence or presence of SRP (or SRP(68/72^N)), which could potentially complicate the interpretation of the targeting assay. For this reason, a targeting assay was also performed in which SRP was added after translation (Figure 6B). Nascent chains were allowed to accumulate, cycloheximide was added, and the translation extract was divided. Samples were then incubated either in the absence or presence of SRP prior to the addition of membranes and fractionation by sedimentation. In this case the nascent chain distribution in each of the targeting assays was identical, since SRP was absent during their synthesis.

In the absence of SRP (Figure 6, lanes a and b), most of the chains were found in the supernatant. In the presence of SRP (lanes c and d), most of the chains (~80% of the chains in [A] and ~60% of the chains in [B]) were targeted to the membrane. When SRP(68/72^N) was included in the assay (lanes e and f), a distribution similar to that seen in the absence of SRP was found, i.e., most of the chains were found in the supernatant.

To summarize, we found that the targeting of the preprolactin nascent chain was markedly reduced. This was true whether SRP was present during the synthesis of the nascent chain or only added after further elongation was inhibited, or whether the targeting assay was performed in high salt (500 mM; not shown), physiological salt (140 mM; Figure 6), or low salt (50 mM; not shown), suggesting that the reaction is blocked at the initial stage of targeting, i.e., in the interaction of SRP with SRP receptor.

We wanted to test whether this particle was in fact hindered in its ability to bind to SRP receptor, and hence we established a system in which to perform SRP receptor affinity chromatography.

SRP(68/72^N) Binds to an SRP Receptor Column with Reduced Affinity

Monoclonal antibodies have been generated against the α-subunit of SRP receptor (SRα) (Tajima et al., 1986). These antibodies have been useful in purifying SRP receptor from preparations of solubilized membranes. Attempts to use these antibodies to block the arrest releasing activity of SRP receptor have failed, suggesting that these antibodies bind to a region of the receptor molecule that is not important for its interaction with SRP. We therefore used these antibodies to generate an SRP receptor column. First, anti-SRα antibodies were coupled to CNBr activated Sepharose. Then this resin was mixed with solubilized membranes under conditions that have been shown to immunopurify SRP receptor. Such a receptor–antibody complex is stable to high salt and thus is a reasonable way to couple SRP receptor to a resin. It has advantages over coupling the receptor directly to the resin: first, such a preparation has never been subjected to harsh conditions, such as the 4.5 M $MgCl_2$ elution step used to immunopurify receptor (Tajima et al., 1986), nor to the lengthy purification procedure used to isolate the complex using SRP-Sepharose chromatography (Gilmore et al., 1982). Second, each receptor molecule on this column is identical, since it is bound to a monoclonal antibody.

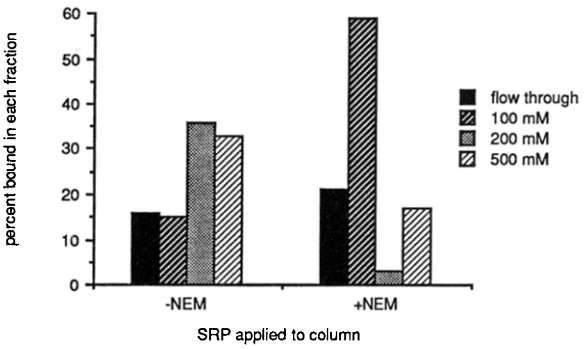

Figure 7. Binding of SRP and SRP(68/72^N) to an SRP-Receptor Column

SRP receptor was bound to anti-SRα Sepharose as described in Experimental Procedures. First SRP and then SRP(68/72^N) was bound and eluted from this column. Binding conditions were 50 mM KOAc, 2 mM $Mg(OAc)_2$. Elution was performed with steps of increasing ionic strength. The percent recovered in each step was calculated by densitometer scanning of a Western blot probed with an anti-68 kd polyclonal serum and ^{125}I-protein A. The total integrated area in the two experiments in arbitrary units were identical for SRP and SRP(68/72^N) (654 and 695, respectively).

If the receptor were directly coupled to CNBr activated Sepharose, it would be coupled in a number of different positions, some of which may influence binding of SRP to the column.

SRP and SRP(68/72^N) were bound and eluted from this SRP receptor column with various steps. The elution profiles for SRP and SRP(68/72^N) are shown in Figure 7. When unmodified SRP was bound to the column, approximately equal amounts were eluted at 200 mM and 500 mM KOAc. This profile is identical to that found when SRP receptor was bound and eluted from an SRP Sepharose column (Gilmore et al., 1982). In contrast, when SRP(68/72^N) was bound to the column, the majority of the particle eluted at 100 mM KOAc. We conclude from this result that SRP(68/72^N) is diminished in its ability to interact with SRP receptor. The small fraction of SRP(68/72^N) eluting at 500 mM KOAc probably reflects incomplete modification by NEM.

We also wished to test whether the 68/72 kd protein could interact with SRP receptor directly. When we passed a mixture of SRP proteins (in the absence of RNA) over the column (not shown), we found that the 68/72 kd protein was specifically depleted from the flow-through fraction. However, because of the intrinsically "sticky" nature of p68/72, we have been unable to demonstrate that this interaction is specific.

SRP(54^N) Has Lost Its Ability to "Recognize" Signal Sequences

We were interested in the reason for the loss of both elongation arrest and translocation promoting activities when the 54 kd moiety of SRP is modified. Because the 54 kd protein has been implicated in signal recognition by virtue of the cross-linking of this protein to signal sequences (Kurzchalia et al., 1986; Krieg et al., 1986), one explanation for this inactivation might be a loss of the signal recog-

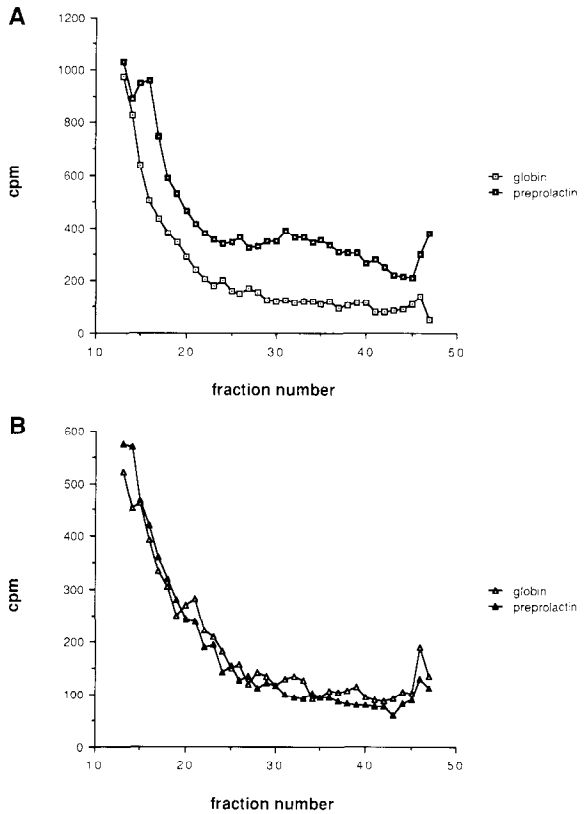

Figure 8. Binding of SRP and SRP(54^N) to Polysomes Synthesizing Globin and Prolactin

^{125}I-labeled SRP or SRP(54^N) was included in translation reactions programmed with total reticulocyte RNA (referred to as "globin" in the figure; open squares and open triangles) or synthetic prolactin mRNA (filled squares and filled triangles). After allowing synthesis for 15 min, samples were layered on top of 13 ml 10%–30% sucrose gradients and spun at 39,000 rpm in a Beckman SW40 rotor for 2 hr. The gradients were fractionated into 0.25 ml fractions. The first fraction (numbered from the top of the gradient) shown on these curves is fraction number 13. (A) SRP. (B) SRP(54^N).

nition activity of the particle. We tested this hypothesis by performing a "polysome binding experiment" (Walter and Blobel, 1981a). SRP and SRP(54^N) were labeled with ^{125}I-Bolton–Hunter reagent and then added to a translation reaction synthesizing either globin or preprolactin. The amount of SRP bound to ribosomes translating these mRNAs was determined by sedimenting such a translation reaction in a sucrose gradient, and determining the amount of ^{125}I-SRP in the polysome region of the gradient. The result of this analysis is shown in Figure 8.

When SRP was added to a translation reaction synthesizing preprolactin (Figure 8A), the amount of SRP found in both the monosome (fractions 15 and 16) and polysome region of the gradient was greater than that found when SRP was added to a translation reaction synthesizing globin. This increase reflects an increase in affinity of SRP for the translating ribosome (Walter and Blobel, 1981a). When SRP(54^N) was added to such translation reactions (Figure 8B), no such increase in SRP in these regions of the gradient was found. We conclude from this result that SRP(54^N) is no longer able to respond to the presence of a signal sequence on the ribosome, and that without this signal recognition event, both elongation arrest and translocation promotion are abolished.

Discussion

Signal recognition particle is composed of a number of different components and exhibits a number of different activities. We have sought to understand the relationship between the structure of SRP and its functions, and for this reason we embarked upon a project of biochemical mutagenesis.

We succeeded by single omission and nuclease digestion experiments in assigning the elongation arrest activity of SRP to a specific domain comprised of the Alu-like sequences of the RNA and the 9/14 kd protein. A particle lacking both these components, SRP(S), was fully active in promoting translocation, and thus retained both signal recognition and translocation promoting activity. A further analysis of functions within the S region of SRP by these methods failed. Similarly, attempts to analyze the function of ribosomes (Nierhaus, 1980; Nomura and Held, 1981; Held et al., 1973; Schulze and Nierhaus, 1982; Tate et al., 1983), by single omission experiments were difficult to interpret. Many different proteins, when omitted from the assembly, affected the same function, and conversely, single omissions often affected a number of different functions. It was never clear in these experiments whether the activity tested in fact resided in a domain built up from a number of different proteins or whether the overall structure of the ribosome was severely altered (yielding, for example, active sites in the wrong geometry). Similar criticisms could be made regarding single omission experiments performed on SRP.

It was with the caveats of single omission experiments in mind that we designed a scheme for modifying single protein components of SRP with the sulfhydryl reactive agent NEM. We felt that such an inactivation procedure would yield particles that were assembled approximately normally, and we were encouraged by the result that NEM modification did not cause the disassembly of SRP (Figure 1).

One incentive for using the reagent NEM in these experiments was that each polypeptide had a site that could potentially be modified, as was seen when the isolated proteins were modified with ^{3}H-NEM (Figure 2). However, some of these sites of modification appear to have been protected when RNA was allowed to bind to the protein prior to modification. Most strikingly, the 9 kd protein, which was labeled rather strongly in isolation (lane a), was almost completely protected from modification when the RNA was present (lane e). There are several possible reasons for this protection. One is that the sulfhydryl groups lie along an RNA binding region of the protein, and the RNA is directly protecting these groups. A second is that the RNA stabilizes the protein in a conformation that sequesters the sulfhydryl groups. It is reasonable to con-

Table 2. Summary of "Mutants" Obtained by Removal or Alteration of Specific Protein or RNA Domains within SRP

Mutant Phenotype	Particle Displaying the Phenotype
Signal Recognition(+) Translocation(+) Elongation Arrest(−)	SRP(−9/14),[a] SRP(S)[b]
Signal Recognition(+) Translocation(−) Elongation Arrest(+)	SRP (68/72^N)
Signal Recognition(−)[c] Translocation(−) Elongation Arrest(−)	SRP(54^N)

[a] Siegel and Walter, 1985.
[b] Siegel and Walter, 1986.
[c] Because signal recognition is a prerequisite for both elongation arrest and protein translocation, the particle lacking this function is completely inactive (see Discussion).

clude from the protection that the 9 kd protein contains an RNA binding domain.

Because of the potential for interfering with assembly of the particle by NEM modifying the proteins in isolation, our scheme entailed reconstituting the proteins onto the RNA prior to modification. Using this approach, we were able to generate biochemically every "mutant" we could have hoped for in a truly genetic screen (see Table 2). In addition to the elongation arrest defective phenotype we had isolated earlier, we generated particles that were active in elongation arrest, but could not promote translocation, and ones that had lost both activities, which we show here to be the result of a signal recognition defective phenotype. In other words, each of the activities of SRP that we have measured can be destroyed by removing or altering a protein component.

The picture that we get from this series of experiments is that the activities of the particle reside in the protein domains, and that the RNA serves primarily as a structural lattice, holding the proteins in specific geometry. It should be noted, however, that a similar picture would have been seen for RNAase P (Guthrie and Athison, 1980; Kole and Altman, 1979) in which it was later shown that the RNA contains the enzymatic activity of the particle (Guerrier et al., 1983). Only when electrostatic repulsion of the two RNAs was diminished (by the inclusion of high concentrations of spermidine in the reaction) was it possible to uncover the enzymatic activity of the RNA alone. These ionic conditions are incompatible with the standard translocation assay (because they would inhibit protein synthesis). Using the assay in which SRP-mediated targeting is uncoupled from elongation (Figure 6B), we attempted to uncover an activity for SRP RNA alone; preliminary experiments performed under high concentrations of spermidine failed to uncover such an activity (not shown).

Signal Recognition, Elongation Arrest, and Translocation Promotion Are Independent Activities

We found that SRP(−9/14) and SRP(68/72^N) have converse phenotypes. SRP(−9/14) lost the ability to arrest elongation but could still promote translocation, while SRP(68/72^N) lost the ability to promote translocation but could still arrest elongation. Both these particles must still be able to recognize signal sequences. We concluded from this that signal recognition can be uncoupled from elongation arrest, and that signal recognition can be uncoupled from translocation promotion. Therefore, it follows that all three activities are distinct.

p68/72 Interacts with SRP Receptor

Microsomal membranes fail to release the arrest induced by SRP(68/72^N) (Figure 4D), demonstrating that SRP receptor cannot interact functionally with this SRP and cause its release from the ribosome. The continued arrest of preprolactin by SRP(68/72^N) does not explain the translocation defective phenotype because removal of the elongation arrest domain does not restore translocation (Figure 5). Rather, nascent chains of preprolactin bound to SRP(68/72^N) fail to be targeted to microsomal membranes under any salt conditions (Figure 6), suggesting that the initial interaction of the nascent chain-ribosome-SRP intermediate with SRP receptor did not occur. Finally, NEM inactivation of p68/72 reduced the binding of SRP to an SRP receptor affinity column (Figure 7). Together, these data strongly support the hypothesis that p68/72 interacts directly with SRP receptor to target secretory proteins to the ER membrane.

p54 Is Required for Signal Recognition

When we tested SRP(54^N) for its ability to bind to ribosomes synthesizing preprolactin, we found that the affinity was not detectably increased over its affinity for ribosomes synthesizing globin (Figure 8). Such a result suggests that p54 is required for signal recognition per se. This result is consistent with the finding that p54 and the signal sequence can be specifically cross-linked (Kurzchalia et al., 1986; Krieg et al., 1986).

Scoulica et al. (1987) found that treatment of SRP with 10 μg/ml elastase completely inactivated the particle and resulted in the release of large protein domains from both p72 and p54. We think it likely that the signal recognition domain of p54 has been released by this procedure, and that this in turn is responsible for the inactivation of SRP. By assembling SRPs in which only p54 is digested (using methods similar to the ones described here), this hypothesis could be tested directly.

It is intriguing that the 54 kd protein, which is absolutely essential for the activity of SRP, is the one that is associated least tightly in the particle. It does not bind directly to the RNA either by cosedimentation (Walter and Blobel, 1983a) or footprint analysis (Siegel and Walter, unpublished data); rather it requires the 19 kd protein for its association with SRP. The lack of a direct association of p54 with the RNA may reflect the evolution of p54 from a peripheral membrane protein that acted directly as a signal receptor to a component of a ribonucleoprotein that interacted with ribosomes, or it may reflect a multiplicity of p54s that have specificities for different classes of signal sequences. There are certainly other possible explanations for this finding, the resolution of which awaits further study.

In summary, the use of NEM to selectively inactivate single proteins in the signal recognition particle allowed us to map the functions of SRP to particular protein domains. Such a method may be useful in the study of the structure and function of ribonucleoproteins and other complex biological structures.

Experimental Procedures

Materials

^{125}I-Bolton–Hunter reagent (diiodo form, 4000 Ci/mmol) was purchased from Amersham; ^{125}I-protein A was from ICN; ^{3}H-N-ethylmaleimide (40 Ci/mmol) from NEN; N-ethylmaleimide, glutathione and HEPES from Sigma; other reagents as described previously (Siegel and Walter, 1985).

Purification of SRP and SRP Proteins

SRP was purified according to Walter and Blobel (1983b) and was frozen after the DEAE concentration step. SRP was disassembled as in Siegel and Walter (1985) with the following modification. The ionic strength of the SRP fraction was determined by conductivity measurements and adjusted to 250 mM potassium acetate (KOAc), 5 mM EDTA prior to disassembly. Proteins derived from 1 mg of SRP could be purified using a 1 ml hydroxylapatite column, followed by three 50 μl CM Sepharose columns. DTT was omitted from the wash and elution buffers of the CM Sepharose columns. 10% glycerol was included in these buffers as a cryoprotectant and to protect fror hydroxyl radical damage.

Reconstitution of Partial SRPs

Reconstitution was performed as described (Walter and Blobel, 1983a). Reconstitution buffer (SRP buffer) consisted of 20 mM HEPES (pH 7.5), 500 mM KOAc, 5 mM magnesium acetate ($Mg[OAc]_2$), 0.01% Nikkol. DTT was generally omitted from the reconstitution reaction.

Labeling of SRP and Reconstitutes with ^{125}I-Bolton–Hunter Reagent

Five hundred microCuries diiodo-Bolton–Hunter reagent was transferred to 50 μl SRP buffer. Ten microliters of this solution was then added to 5 pmol of SRP. The mixture was incubated for 2 hr at 4°C before the addition of Tris (pH 8.0) to 100 mM to stop the reaction. Because ^{125}I-SRP was previously shown to bind irreversibly to columns when gel filtration was employed to separate labeled material from unincorpated Bolton–Hunter reagent (Siegel and Walter, 1985), labeled SRP was purified by sucrose gradient sedimentation. A one milliliter gradient was formed containing steps of 5%, 10%, 15%, and 20% sucrose in SRP buffer and allowed to diffuse overnight at 4°C. The gradients were spun in the TLS-55 swinging bucket rotor in the Beckman TL-100 Tabletop Ultracentrifuge for 3 hr at 55,000 rpm (g_{av} = 201,247 g). The gradients were fractionated by hand into 75 μl fractions, and the peak visualized following resolution of the proteins by PAGE in SDS using autoradiography on X-ray film.

Labeling of SRP Proteins and Reconstitutes with ^{3}H-NEM

SRP proteins were reconstituted in the presence of a 10-fold molexcess of SRP RNA. Sixty microCuries^{3}H-NEM in pentane was mixed with 5 pmol SRP proteins or reconstitutes and the pentane evaporated out of the mixture using a gentle stream of N_2 gas. The NEM modification was then performed as above. After labeling, the samples were TCA precipitated and resolved by polyacrylamide gel electrophoresis.

Generation of SRPs in which a Single Protein Is Modified with NEM

The protein to be modified (2 μl of a 2.5 μM solution in 2× SRP buffer) was first mixed with a 2-fold molar excess of SRP RNA (2 μl 5 μM in H_2O) and reconstituted. If protein alone was modified, H_2O was added in place of RNA. Then 1 μl NEM in SRP buffer was added to a final concentration of 1 mM. After 30 min at 25°C, 1 μl 50 mM DTT or 1 μl 50 mM glutathione (both in SRP buffer) was added to quench any unreacted NEM. In control reactions DTT or glutathione was added prior to the addition of NEM. All remaining SRP components were then added in SRP buffer to a final volume of 10 μl and a final concentration of 1 μM. The reconstitution reaction (10 min on ice, 10 min 37°C) was then repeated.

In Vitro Transcription

Plasmid pSPBP4 encoding bovine preprolactin was constructed by Dr. W. B. Hansen in our laboratory in the following manner. Plasmid pSP64T, which contains the 5′ and 3′ untranslated regions of the Xenopus β-globin gene separated by a BglII linker was obtained from Doug Melton (Harvard University). These regions were cloned between the HindIII and PstI sites of the vector pSP64 marketed by ProMega Biotec. The BglII site of pSP64T was filled in with Klenow DNA polymerase and converted to an NcoI site which conforms to the optimal initiation context (ACCATGG; Kozak, 1986). The resulting vector was cleaved with NcoI and PstI and then the NcoI (partial digest)-PstI fragment from the bovine prolactin cDNA clone pBPRL 72 (Sasavage et al., 1982) was inserted. In this construction, the 5′ untranslated region of preprolactin has been deleted; the 3′ untranslated region, poly(A) and poly(G) tails have been retained. pSPBP4 was cut with EcoRI and transcribed with SP6 polymerase according to Hansen et al. (1986), with a 5:1 ratio of GpppG to GTP to cap the 5′ end. Transcription was for 1 hr at 40°C. The transcription reaction was then frozen in liquid nitrogen and stored at −80°C until use.

In Vitro Translations

Translations were performed as described previously (Erickson and Blobel, 1983) with the following modifications. Wheat germ extract was prespun for 2 min at 30 psi in the Beckman airfuge A-110 rotor to remove contaminating membranes. Translation reactions contained 0.1 U/μl RNAse inhibitor and 8 μM S-adenosyl methionine. The ionic conditions of this assay were kept at 144 mM KOAc and 3 mM $Mg[OAc]_2$.

Activity Assays

Elongation arrest and translocation promoting activities were assayed as described previously (Siegel and Walter, 1985) with the following modifications. Synthetic preprolactin mRNA was used in place of bovine pituitary RNA. Translations were performed in a final volume of 10 μl.

SRP was titrated into the translation reaction in the absence or presence of 1 equivalent (eq, defined in Walter et al., 1981) SRP-depleted membranes per 10 μl to measure elongation arrest and translocation, respectively. The amount of preprolactin, prolactin, and globin in each lane was quantitated using an LKB Ultroscan XL Laser densitometer (LKB Instruments, Gaithersberg, MD). The amount of elongation arrest was calculated as before (Siegel and Walter, 1985), but the amount of translocation was calculated according to Garcia et al. (1987), so that the inhibition of preprolactin synthesis by SRP(68/72^{N}) would not give abberantly high numbers for percent processing:

$$\%\ \text{translocation(a)} = \frac{(8/7)\text{prolactin(a)} \times \text{globin(0)} \times 100}{\text{globin(a)} \times [\text{preprolactin(0)} + (8/7)\text{prolactin(0)}]}$$

Elongation arrest in the presence of membranes was calculated using the following equation:

$$\text{percent inhibition(a)} = 100 - \frac{[\text{preprolactin(a)} + (8/7)\text{prolactin(a)}] \times \text{globin(0)} \times 100}{\text{globin(a)} \times [\text{preprolactin(0)} + (8/7)\text{prolactin(0)}]}$$

Cotranslational targeting assays (Connolly and Gilmore, 1986) were performed by adding SRP to 120 nM in a 50 μl translation. The translation reaction was allowed to incubate for 15 min at 26°C to allow the accumulation of arrested chains. Then cycloheximide was added to 1 mM to block further elongation. Fifteen microliters of the reaction was mixed with 4 eq of SRP depleted membranes and allowed to incubate for 5 min at 22°C. Stability of the targeted chains to high salt was tested by adding KOAc and $Mg(OAc)_2$ to 500 mM and 5 mM final, respectively. The mixture was layered over a 50 μl cushion comprised of 250 mM sucrose, 20 mM HEPES, 1 mM DTT and either 140 mM KOAc, 2.5 mM $Mg(OAc)_2$ (physiological salt), or 500 mM KOAc, 5 mM $Mg(OAc)_2$ (high salt). Samples were spun for 3 min (physiological salt) or 4 min (high salt) at 20 psi in the Beckman A-100/30 rotor in the Beckman airfuge, not counting acceleration or deceleration times. The entire supernatant, including the cushion, was added to 250 μl 2% CTABr. Like-

wise, the pellet was dissolved in 250 μl 2% CTABr. Then 50 μg calf liver tRNA and 250 μl 0.5 M sodium acetate (NaOac) (pH 5.4) were added, and the samples were incubated for 10 min at 30°C. Samples were spun in a microfuge for 5 min, the pellets rinsed twice with 500 μl acetone/HCl, dried in a Speed-Vac, and resuspended in 25 μl SDS-PAGE sample buffer. After 1 hr at 55°C, nascent chains were resolved by SDS-PAGE, the gels were fluorographed (Siegel and Walter, 1985), and the bands visualized by autoradiography.

"Posttranslational" targeting assays were performed as for the cotranslational assay with the following exception. Nascent chains were allowed to achieve "steady state" by incubation of the translation reaction at 22°C for 14 min prior to addition of cycloheximide to 1 mM. A single translation reaction was used for all the assays. The translation reaction was divided and SRP or an SRP derivative was added to 80 nM and incubated for 10 min at 22°C. The remainder of the assay was as above.

SRP Receptor Affinity Chromatography

A monoclonal antibody against the α-subunit of SRP receptor (Tajima et al., 1986) was coupled to CNBr activated Sepharose as described. Ten milliliters of solubilized membranes (~1 eq per μl, representing a total of about 1 nmol SRα) was mixed with 0.2 ml of resin. After 2 hr of mixing in batch, a column was poured from this resin, which was then washed with 2 ml of buffer A (20 mM HEPES [pH 7.4], 0.5 mM glutathione, 0.5% Nikkol) containing 500 mM KOAc and 5 mM $Mg(OAc)_2$. The column was then washed with 2 ml of Buffer A containing 50 mM KOAc, 2 mM $Mg(OAc)_2$ before an SRP preparation (7.5 pmol) was bound to it. These preparations were adjusted to a buffer composition equal to the 50 mM KOAc wash buffer (a 10-fold dilution) and then loaded onto the SRP receptor–antireceptor Sepharose column. One milliliter of load buffer followed and was pooled with the flow-through fraction. The column was eluted with 1 ml each of buffer A containing the following concentrations of potassium and magnesium: 100 mM KOAc, 2 mM $Mg(OAc)_2$; 200 mM KOAc, 2 mM $Mg(OAc)_2$; and 500 mM KOAc, 5 mM $Mg(OAc)_2$. One milliliter of the 50 mM KOAc wash buffer was then passed over the column to prepare it for the next sample to be loaded.

Western Blotting

After resolution of the protein fractions by SDS-PAGE, the proteins were transferred to nitrocellulose using 0.75 Amp current for 2 hr. The nitrocellulose was blocked with 4% BSA in 10 mM sodium phosphate buffer (pH 7.5), 150 mM NaCl, 1 mM EGTA, 0.2% TX-100, 0.02% NaN_3 (BSA buffer) overnight. The blot was then incubated with a polyclonal serum against the 68 kd protein diluted 1:200 in BSA buffer for 6 hr. The blot was washed 5 × 3 min with 50 mM triethanolamine-HCl (pH 7.5), 100 mM NaCl, 2 mM EDTA, 0.5% TX-100, 0.1% SDS (SDS buffer), rinsed with BSA buffer, and then incubated with BSA buffer for 5 min. ^{125}I-Protein A (2.5 μCi) in 50 ml BSA buffer was incubated with the blot for 30 min. The blot was rinsed again 5 × 3 min in SDS buffer, three times with Tris buffered saline (25 mM Tris-HCl [pH 8.0], 144 mM NaCl), and blotted dry. Exposure was for 24 hr with an intensifying screen. The amount of 68 kd protein in each fraction was quantitated by densitometry using a Zeineh Soft Laser Scanning densitometer (Biomed Instruments Inc., Fullerton, CA).

Polysome Binding Analysis

Twenty-four microliters of gradient purified ^{125}I-SRP or ^{125}I-SRP(54^N) (~200 fmol) was included in a 100 μl wheat germ translation reaction programmed with either total reticulocyte RNA or synthetic preprolactin mRNA and incubated for 15 min at 26°C. The samples were then loaded onto 13 ml 10%–30% sucrose gradients (50 mM triethanolamine-OAc [pH 7.5], 100 mM KOAc, 2.5 mM $Mg(OAc)_2$, 1 mM DTT, 10 μg/ml insulin) that were poured in gelatin-coated polyallomer SW40 ultracentrifuge tubes. Gradients were spun for 2 hr at 39,000 rpm (Acc 7 Dec 7) at 4°C. Gradients were fractionated by underlayering with 60% sucrose using an Isco gradient fractionator. Forty-nine 0.25 ml fractions were collected from each gradient. Fractions were counted with a Beckman 4000 gamma counter.

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References

Connolly, T., and Gilmore, R. (1986). Formation of a functional ribosome-membrane junction requires the participation of a GTP-binding protein. J. Cell Biol. *103*, 2253–2261.

Erickson, A. H., and Blobel, G. (1983). Cell-free translation of messenger RNA in a wheat germ system. Meth. Enzymol. *96*, 38–50.

Garcia, P. D., Ghrayeb, J., Inouye, M., and Walter, P. (1987). Wild type and mutant signal peptides of Escherichia coli outer membrane lipoprotein interact with equal efficiency with mammalian signal recognition particle. J. Biol. Chem. *262*, 9463–9468.

Gilmore, R., and Blobel, G. (1983). Transient involvement of signal recognition particle and its receptor in the microsomal membrane prior to protein translocation. Cell *35*, 677–685.

Gilmore, R., Walter, P., and Blobel, G. (1982). Protein translocation across the endoplasmic reticulum. II. Isolation and characterization of the signal recognition particle receptor. J. Cell Biol. *95*, 470–477.

Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell *35*, 849–857.

Guthrie, C., and Atchison, R. (1980). Biochemical characterization of RNAse P: a tRNA processing activity with protein and RNA components. In Transfer RNA: Biological Aspects, J. Abelson, P. Schimmel, and D. Soell, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 83–97.

Hansen, W., Garcia, P. D., and Walter, P. (1986). In vitro protein translocation across the yeast endoplasmic reticulum: ATP-dependent post-translational translocation of the prepro-α-factor. Cell *45*, 397–406.

Held, W. A., Mizushima, S., and Nomura, M. (1973). Reconstitution of Escherichia coli 30S ribosomal subunits from purified molecular components. J. Biol. Chem. *248*, 5720–5730.

Hobden, A. H., and Cundliffe, E. (1978). The mode of action of alpha sarcin and a novel assay for the puromycin reaction. Biochem. J. *170*, 57–61.

Kozak, M. (1986). Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. Cell *44*, 283–292.

Krieg, U. C., Walter, P., and Johnson, A. E. (1986). Photocrosslinking of the signal sequence of nascent preprolactin to the 54 kd polypeptide of the signal recognition particle. Proc. Natl. Acad. Sci. USA *83*, 8604–8608.

Kurzchalia, T. V., Wiedmann, M., Girshovich, A. S., Bochkareva, E. S., Bielka, H., and Rapoport, T. A. (1986). The signal sequence of nascent preprolactin interacts with the 54K polypeptide of the signal recognition particle. Nature *320*, 634–636.

Mueckler, M., and Lodish, H. F. (1986). The human glucose transporter can insert posttranslationally into microsomes. Cell *44*, 629–637.

Nierhaus, K. H. (1980). Analysis of the assembly and function of the 50S subunit from Escherichia coli ribosomes by reconstitution. In Ribosomes: Structure, Function, and Genetics, G. Chambliss, G. R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura, eds. (Baltimore: University Park Press), pp. 267–294.

Nomura, M., and Held, W. A. (1974). Reconstitution of ribosomes: studies of ribosome structure, function and assembly. In Ribosomes, M. Nomura, A. Tissieres, and P. Lengyel, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 193–223.

Perara, E., Rothman, R. E., and Lingappa, V. R. (1986). Uncoupling translocation from translation: implications for transport of proteins across membranes. Science *232*, 348–352.

Sasavage, N. L., Nilson, J. H., Horowitz, S., and Rottman, F. M. (1982). Nucleotide sequence of bovine preprolactin messenger RNA. Evidence for sequence polymorphism. J. Biol. Chem. *257*, 678–681.

Scoulica, E., Kravse, E., Meese, K., and Dobberstein, B. (1987). Disassembly and domain structure of the proteins in the signal-recognition particle. Eur. J. Biochem. *163*, 519–528.

Siegel, V., and Walter, P. (1985). Elongation arrest is not a prerequisite for secretory protein translocation across the microsomal membrane. J. Cell Biol. *100*, 1913–1921.

Siegel, V., and Walter, P. (1986). Removal of the Alu structural domain from signal recognition particle leaves its protein translocation activity intact. Nature *320*, 81–84.

Tajima, S., Lauffer, L., Rath, V. L., and Walter, P. (1986). The signal recognition particle receptor is a complex that contains two distinct polypeptide chains. J. Cell Biol. *103*, 1167–1178.

Tate, W. P., Schulze, H., and Nierhaus, K. H. (1983). The Escherichia coli ribosomal protein L11 suppresses release factor 2 but promotes the release factor 1 activities of peptide chain termination. J. Biol. Chem. *258*, 12816–12820.

Ullu, E., Murphy, S., and Melli, M. (1982). Human 7SL RNA consists of a 140 nucleotide middle-repetitive sequence inserted in an Alu sequence. Cell *29*, 195–202.

Walter, P., and Blobel, G. (1980). Purification of a membrane-associated protein complex required for protein translocation across the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA *77*, 7112–7116.

Walter, P., and Blobel, G. (1981). Translocation of proteins across the endoplasmic reticulum. III. Signal recognition protein (SRP) causes signal sequence and site specific arrest of chain elongation that is released by microsomal membranes. J. Cell Biol. *91*, 557–561.

Walter, P., and Blobel, G. (1982). Signal recognition particle contains a 7S RNA essential for protein translocation across the endoplasmic reticulum. Nature *299*, 691–698.

Walter, P., and Blobel, G. (1983a). Disassembly and reconstitution of signal recognition particle. Cell *34*, 525–533.

Walter, P., and Blobel, G. (1983b). Signal recognition particle: a ribonucleoprotein required for cotranslational translocation of proteins, isolation and properties. Meth. Enzymol. *96*, 682–691.

Walter, P., Ibrahimi, I., and Blobel, G. (1981). Translocation of proteins across the endoplasmic reticulum. I. Signal recognition protein (SRP) binds to in vitro assembled polysomes synthesizing secretory proteins. J. Cell Biol. *91*, 545–550.

Walter, P., Gilmore, R., and Blobel, G. (1984). Protein translocation across the endoplasmic reticulum. Cell *38*, 5–8.