Translocation of Preproinsulin Across the Endoplasmic Reticulum Membrane

The Relationship Between Nascent Polypeptide Size and Extent of Signal Recognition Particle-Mediated Inhibition of Protein Synthesis

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Signal recognition particle (SRP) induces elongation arrest of nascent presecretory proteins as the signal peptide protrudes from the large ribosomal subunit. To examine the relationship between the size of the precursor and extent of SRP-mediated inhibition of polypeptide chain elongation, we performed in vitro translation experiments in the presence of SRP using a series of truncated preproinsulin mRNA molecules.

These precursors possessed the same NH₂ terminus as native preproinsulin followed by progressively shorter COOH termini. SRP inhibited translation of precursors as short as 64 amino acids in length, however, the extent of inhibition diminished for shorter precursors. This correlated with a reduction in the time required for ribosomes to transit through the mRNA encoding the shortened precursors. By exploiting a chimeric protein comprising the first 71 residues of preproinsulin fused to the bacterial cytoplasmic enzyme chloramphenicol acetyltransferase, we demonstrate that the signal peptide is a reversible process even in the absence of microsomal membranes, and that SRP can arrest polypeptide chain elongation at multiple stages during translation.

Eukaryotic secretory proteins are targeted to and translocated across the endoplasmic reticulum (ER) membrane because they possess a signal peptide, or its functional equivalent, that mediates interaction with the secretory apparatus. Typical signal sequences of 20–25 amino acids in length are located at the amino terminus of the protein and usually possess an NH₂-terminal positively charged domain followed by a region of predominantly hydrophobic amino acids and terminate with small neutral residues of higher average polarity than those in the hydrophobic core (Von Heijne, 1985; Giersch, 1989). In the first step of the translocation process, signal recognition particle (SRP), a ribonucleoprotein complex consisting of six proteins (72, 68, 54, 19, 14, and 9 kDa) with a 7S RNA, binds to the nascent signal peptide as it emerges from the ribosome, and transiently arrests translation (Walter and Blobel, 1981a, 1981b; Walter and Lingappa, 1986). The SRP-ribosome-nascent chain complex is targeted to the ER by virtue of the affinity between SRP and SRP receptor (or docking protein) in the ER membrane (Gilmore et al., 1982a, 1982b; Meyer et al., 1982; Gilmore and Blobel, 1983). The SRP receptor displaces SRP from the ribosome-nascent chain complex in a GTP-dependent reaction (Connolly and Gilmore, 1989), thereby releasing translation arrest and permitting the co-translational translocation of the secretory polypeptide through the membrane. Signal peptide binding, elongation arrest, and SRP receptor binding are mediated by different components of SRP. The 54-kDa protein, which was shown to possess a GTP-binding motif as well as a methionine-rich domain (Romisch et al., 1989; Bernstein et al., 1989), is the SRP component that binds directly to the signal peptide. An alkylated 54-kDa subunit loses its signal peptide binding capability (Siegel and Walter, 1988), and can be cross-linked to generate signal peptide-54 kDa protein complexes (Wiedmann et al., 1987). The presence of methionine-rich putative amphipathic α-helices in the 54-kDa polypeptide (Bernstein et al., 1989) are consistent with earlier observations that signal peptide binding relies on hydrophobic interactions that are disrupted if hydrophilic analogs of hydrophobic amino acids are incorporated into the signal peptide (Walter and Blobel, 1981b). Elongation arrest is mediated by the 9/14-kDa heterodimer; SRP devoid of these components binds nascent polypeptides but does not arrest translation (Siegel and Walter, 1985). In the absence of its elongation arrest capacity SRP mediates translocation less efficiently than normal, either because the nascent chain is elongated into a translocation-incompetent state or is completed prior to targeting to the ER membrane. SRP does not bind the signal peptide of proteins not tethered to a ribosome (Wiedmann et al., 1987), and therefore is unable to target completed proteins to the ER membrane.

Lipp et al. (1987) observed that for some presecretory proteins, translation in the presence of SRP but absence of membranes (i.e. no SRP-receptor) generates a range of nascent chains whose average size increases with time until the full-length polypeptide is produced. These results were interpreted as demonstrating a reversible interaction between SRP
and the nascent chain, with elongation continuing during the off-phase of binding. Rapoport et al. (1987) proposed that nascent chains have an SRP window, defined as "the distance between the first and last site of the polypeptide chain that can interact with SRP." When the nascent chain is elongated beyond the SRP window, SRP no longer binds, either because the nascent chain assumes a conformation in which the signal sequence is sequestered in the interior of the molecule where SRP cannot bind, or the distance between the protruded signal peptide and the ribosome is too great to be spanned by SRP.

We are interested in the initial stages of the translocation process, i.e. SRP binding and arrest of nascent polypeptide chain elongation. To study these events we have constructed a series of derivatives of preproinsulin, all of which have the same amino terminus as the parent molecule (including the signal peptide) with progressively smaller carboxyl termini. In a previous paper (Okun et al., 1990) we determined that 50 amino acids was the smallest peptide size for which SRP-mediated translational arrest was possible and suggested that the initial SRP binding occurs when only 5–10 residues of the signal peptide protrude from the large ribosomal subunit. Here we test aspects of the reversible signal peptide-SRP model by examining the relationship between the length of a peptide and the extent of SRP-mediated translational inhibition. If the SRP-signal peptide interaction is truly a dynamic on-off process, the nascent chain, with elongation continuing during the on-phase of this cycle, proteins with a reduced SRP window size (i.e. shorter peptides) would have less opportunity to bind SRP and undergo translation arrest. We predicted that the translation of shorter peptides should be less inhibited by SRP than longer polypeptides. We demonstrate here that SRP-mediated inhibition can be detected for a precursor of 64 residues, whereas the extent of inhibition diminished for shorter polypeptides. Our data are consistent with a model that proposes SRP binding to the signal peptide is reversible and that elongation arrest occurs at multiple sites during translation.

**EXPERIMENTAL PROCEDURES**

**Materials**

[^35S]Cysteine at the highest available specific activity was purchased from Amersham/Seaple Chemicals. Restriction enzymes, the Klenow fragment of DNA polymerase I, and human placental RNasin were from Bethesda Research Laboratories, Gaithersburg, MA. BglII linkers were from New England Biolabs, Beverly, MA. *Escherichia coli* RNA polymerase, 7-methylguanosine 5'-monophosphate. To compensate for the reduction in translation efficiency caused by synchonization, the RNA transcribed from the 1.5 µg of plasmid DNA was used to program a 25-µl translation reaction. Microsomal vesicles (RM) were prepared from canine pancreas as described (Walter and Blobel, 1983a), and used at a final concentration of 3.5 A260 units/ml unless otherwise indicated. SRP and ribosomes were removed from microsomes and membranes by treatment with EDTA and high salt (K-RM) (Walter and Blobel, 1983b). SRP was further purified as described (Walter and Blobel, 1983b).

**CTAB Precipitation**—Precipitation of nascent polypeptide chains with hexadecyltrimethylammonium bromide (CTAB) was performed as described previously (Gilmore and Blobel, 1985; Okun et al., 1990).

**RESULTS**

The construction of *in vitro* transcription-translation experiments encoding anglerfish preproinsulin and preproinsulin-derived truncations was as previously described (Okun et al., 1990; also see "Methods"). These molecules contain the preproinsulin signal peptide and different lengths of the B and C chains, and are 78 (ppPUT), 64 (ppAUT), and 45 residues (ppSUT) in size (Fig. 1). The DNA fragments encoding ppPUT and ppAUT were inserted into the pDS5 expression vector (Steuber et al., 1984) downstream of the phase T5-derived promoter and upstream of a universal translation terminator. In addition to preproinsulin sequences, each insert contains either 7 (ppPUT and ppSUT) or 6 (ppAUT) extra amino acids at the COOH terminus encoded by the plasmid (Okun et al., 1990).

All DNA fragments were inserted at the unique EcoRI site of the pDS5 multiple cloning site, so that the *in vitro* transcribed mRNA species encoding these polypeptides possessed identical 5'-untranslated regions. This ensured that the only factor determining the relative translational efficiency of these polypeptides depended solely on differences in their protein coding region.

Our initial experimental protocol to measure SRP inhibi-
Fig. 1. Schematic representation of anglerfish preproinsulin and the carboxyl-terminal truncations. The construction of pDSS-derived in vitro transcription RNA in the presence or absence of SRP, removing aliquots from the translation products at various time intervals, followed by analysis upon SDS-PAGE. Assessing the extent of SRP inhibition for ppPUT and ppAUT in this fashion proved impossible because the completed translation products were so close in size to the SRP-arrested nascent chains that there was no discrete polypeptide which could be resolved by SDS-PAGE and quantitated by densitometric analysis (Okun et al., 1990). This difficulty was circumvented by employing the cationic detergent CTAB, which precipitates nucleic acids, including peptidyl-tRNA (Gilmore and Blobel, 1985; Okun et al., 1990); completed proteins, in contrast, remain in the CTAB supernatant. A control experiment utilizing puromycin-terminated nascent polypeptides was performed that verified the efficacy of CTAB in fractionating peptidyl-tRNA from completed proteins (data not shown).

Initially we measured the time course for in vitro translation of preproinsulin (Fig. 2, panel A). ppPUT (panel C), and ppAUT (panel E) in the absence or presence of SRP. Globin mRNA (the translation of which is unaffected by the presence of SRP) was included as a control for variations in translation unrelated to SRP effects. At early times SRP-mediated inhibition of preproinsulin synthesis was complete (panel A), however, after 30 min, preproinsulin was synthesized even in the presence of SRP (lanes 8–10). The relative levels of preproinsulin synthesized at different time points were quantified via densitometric analysis (panel B); the pattern observed is consistent with transient inhibition by SRP. Initially, SRP binds to nascent chains and blocks protein completion, but since the SRP binding is reversible the arrested chains are essentially completed at later time points. The synthesis of preproinsulin in the presence of SRP did not reach the same level as in the absence of SRP because by late times in translation, initiation activity in the in vitro translation system decays. Translation inhibition for ppPUT (78-mer) is substantially less than for preproinsulin at early time points (panels C and D), presumably because there are fewer potential sites along the smaller nascent chain for SRP to bind and block polypeptide chain elongation. Previous investigators (Walter and Blobel, 1981a, 1981b; see Walter and Lingappa, 1986) estimated that the SRP-arrested nascent chain was between 70 and 80 residues in length. Based on this estimate, we expected that the ppAUT polypeptide (only 64 residues) would be too small to be inhibited in its translation by SRP. However, inhibition of translation of ppAUT was observed (panels E and F), suggesting that 70–80 amino acids was an overestimate of the minimum size necessary for SRP arrest of a nascent polypeptide. Globin mRNA was included in these experiments to serve as a control for variation in overall translation efficiency in the absence and presence of SRP; furthermore, the globin translation products were used as an internal control in the densitometric analysis ("Methods"). Thus, although there appears to be more ppAUT at 60 min than at 30 min in the presence of SRP (panel E, compare lanes 9 and 7), relative to the amount of globin, ppAUT in lane 9 is less intense than at 30 min (lane 7).

Comparison of the relative degree of SRP-mediated inhibition for preproinsulin and the preproinsulin-derived truncations at early and late time points is shown in Fig. 3. The ordinate value is a measure of the extent of SRP-induced inhibition at 30 (open bars) and 75 min (hatched bars), respectively. The differences between preproinsulin in the 78- and 64-mer polypeptides are considerably less marked at the 75-min time point, whereas there was a direct relationship between polypeptide size and inhibition at 30 min. We have previously demonstrated (Okun et al., 1990) that a 45-mer truncation of preproinsulin (ppSUT, Fig. 1) is below the minimum size for SRP arrest; the relative magnitude of inhibition for ppSUT was therefore included as a control. The observed relative magnitude of inhibition for ppSUT is 1.08; implying that the values for the other precursors are accurate to approximately 10%.

To determine the size of the nascent polypeptide chains for each precursor that are tethered to ribosomes in the absence and presence of SRP, the CTAB-precipitable products of each in vitro translation were analyzed (Fig. 4). The CTAB precipitates from the preproinsulin translation (panel A, lanes 1–10) revealed two discrete sizes of nascent chains that accumulated only in the presence of SRP. Some of these approximated the size minimum for SRP inhibition corresponding to the arrested fragment (A.F., bottom of panels), while others were only slightly smaller than full-length preproinsulin (asterisk). This observation of the accumulation of several distinct sizes of nascent chains rather than a continuum of poorly resolved polypeptides is consistent with SRP arresting translation at discrete points during translation. The average size of the CTAB-precipitable material increased during translation (panel A, asterisk). This time-dependent increase in the average size of the nascent chain is also consistent with transient inhibition by SRP. The CTAB-precipitable material from ppPUT translation, 78 amino acids (panel B), also contained the small arrested fragment chains seen with preproinsulin but the larger polypeptides were absent, implying that the large size classes detected during preproinsulin translation were greater than 78 amino acids in size. As with preproinsulin, the average size of the nascent chains increased with time. CTAB-precipitable polypeptides also accumulated during translation of ppAUT (64 residues) in the presence of SRP (panel C). This material was resolved into two size classes, the smaller of which represents the minimum size for SRP-mediated arrest, which we previously estimated to be approximately 50 amino acids (Okun et al., 1990).

We had previously determined (Eskridge and Shields, 1983) that the half-life for preproinsulin synthesis in the wheat germ system was 6 min. It was therefore of interest to measure the time required to complete synthesis of these precursors in the presence and absence of SRP. Ribosome transit time
**FIG. 2.** SRP-mediated translation inhibition of preproinsulin and preproinsulin-derived truncations. Panel A, full-length preproinsulin mRNA (generated by *in vitro* transcription of plasmid pDS5ins) and globin mRNA were translated in the absence (lanes 1–5) or presence (lanes 6–10) of SRP (720 units/ml). At the indicated times, aliquots were removed and treated with 2% CTAB, pelleted in a microcentrifuge for 10 min, and the supernatant material precipitated with an equal volume of ice-cold 20% trichloroacetic acid, followed by centrifugation. The pellets of the trichloroacetic acid-precipitable material were dissolved in SDS gel loading buffer and analyzed by SDS-PAGE using 15% acrylamide gels containing 7 M urea. The migration of preproinsulin (ppins) is indicated. Panel B, the fluorograph in panel A was analyzed densitometrically using an Ultrascan XL laser densitometer (Pharmacia LKB Biotechnology Inc.). Panel C, translation products of 78-mer mRNA; *in vitro* transcribed ppPUT mRNA was co-translated with globin mRNA in the absence (lanes 1–5) or presence (lanes 6–10) of SRP and the translation products analyzed exactly as outlined in panel A. Panel D, densitometric analysis of the 78-mer mRNA translation products shown in panel E. Panel E, translation products of 64-mer mRNA; *in vitro* transcribed ppAUT was co-translated with globin mRNA in the absence (lanes 1–5) and presence (lanes 6–10) of SRP and analyzed as outlined in panel A. Panel F, densitometric analysis of the 64-mer mRNA translation products shown in panel E. Note that the densitometric analysis data (panels B, D, and F) is averaged from two separate experiments. For ppAUT synthesis, the fluorogram shows translation products synthesized up to 75 min of incubation from one experiment, whereas the densitometry includes an averaged 90-min time point. Relative synthesis in the presence of SRP is given by the following:

\[
\frac{\text{Band intensity at } t_{\text{srp}} + \text{SRP}}{\text{Maximum intensity} - \text{SRP}} \times \frac{\text{Intensity of globin} - \text{SRP}}{\text{Intensity of globin} + \text{SRP}} \times 100
\]
through an open reading frame can be experimentally determined by performing synchronous translation reactions, where initiation of protein synthesis occurs within the time window between addition of mRNA and of a 5' cap analog that inhibits further initiation (Rothman and Lodish, 1977). The transit time corresponds to the point in the synchronous translation when the amount of synthesized protein reaches its maximum ($t_{\text{max}}$); this is the time when all the ribosomes that had initiated during synchronization complete synthesis. Synchronous translations of preproinsulin and the truncated polypeptides were performed, and the ribosome transit times were measured in the presence and absence of SRP (Table I).

As expected, SRP induced a time delay in the synthesis of all these polypeptides which was proportional to precursor length. The SRP-induced transit time delay ($\Delta t_{\text{trans}}$) decreased from 16 min for preproinsulin to 0 min for ppAUT. No difference in transit time was observed for ppAUT, even though SRP did inhibit translation and caused accumulation of CTAB-precipitable material. Apparently a high percentage of ribosomes complete synthesis of ppAUT before interacting with SRP, and this background of polypeptides completed within 1 min obscures any SRP-mediated time delay.

Since it has been shown that SRP binds both the ribosome and the signal peptide to inhibit translation (Walter and Blobel, 1981a, 1981b) we determined how large a nascent chain could grow and still be susceptible to SRP-mediated inhibition. For long polypeptide chains, the distance between the ribosome and the signal peptide may become too great for SRP to bind both simultaneously, and consequently, SRP may dissociate from the signal peptide and lose its ability to inhibit translation. To test this idea we translated a chimera consisting of the preproinsulin 78-mer fused to chloramphenicol acetyltransferase, CAT, designated ppPLCAT ($M_\text{r}$, 27,000) (EsKridge and Shields, 1986) in the presence and absence of SRP (Fig. 5), and analyzed the CTAB-precipitable and soluble translation products. If ppPLCAT did not exceed the maximum size for inhibition then a series of SRP-arrested nascent chains ranging in size up to the full-length molecule should be observed. In contrast, if ppPLCAT was larger than the maximum size for inhibition, all arrested polypeptides would be smaller than $M_\text{r}$, 27,000 and the largest would correspond to the maximum size for SRP-mediated inhibition. Examination of the CTAB supernatants (compare lanes 7–12 and 20–25) revealed that translation of ppPLCAT was extensively inhibited by SRP, typical for a protein with a signal peptide. Furthermore, the CTAB-precipitable products synthesized in the presence of SRP were resolved into 4 major species ranging in size from $M_\text{r}$, ~5,000 to ~17,000 (lanes 14–19). The larger polypeptides were considerably less intense than the smaller ones. As predicted, larger nascent chains are less susceptible to SRP inhibition, and for ppPLCAT, the size limit for inhibition is $M_\text{r}$, 17,000.

**DISCUSSION**

The relationship between precursor size and extent of SRP-mediated inhibition observed here is consistent with the molecular models of Lipp et al. (1987) and Rapoport et al. (1987), which postulate that SRP binds the ribosome-nascent chain complex at multiple points during translation and arrests translation in a reversible fashion even in the absence of the SRP receptor. Larger polypeptides offer multiple stages for SRP to bind and arrest translation prior to termination of protein synthesis, hence SRP can undergo multiple on-off cycles before translation is completed. At early times during the synthesis of larger proteins, most ribosomes are halted at one of the multiple potential stages for SRP arrest, explaining the near total inhibition observed for preproinsulin (Fig. 2, panel A) and ppPLCAT (Fig. 5) after 15 and 30 min of incubation. Since SRP binding is reversible, at later times
this inhibition is diminished. For smaller proteins such as the 78-mer (ppPUT) and the 64-mer (ppAUT), inhibition is less marked because these proteins have fewer stages of potential translational arrest.

Our synchronous translation experiments (Table I) show that ribosomal transit time in the presence of SRP becomes progressively shorter as precursor size diminishes and that the transit time of the 64-mer is identical, within the limits of our experimental technique, in the absence or presence of SRP. We suggest that SRP inhibits translational initiation at early time points and that at later times translational inhibition is a consequence of decay in initiation factor activity of the in vitro system. It might be argued that the data from the synchronous translation of ppAUT (Table I) contradict that of Figs. 2 (panels E and F) and 4 (panel C), which show, respectively, SRP-mediated inhibition of ppAUT and accumulation, at early times, of CTAB-precipitable nascent 64-mer. We postulate that in these unsynchronized translations, SRP may have a minimal effect on elongation rate and acts primarily on initiation by "backing-up" ribosomes at the 5'-untranslated region of the mRNA ultimately resulting in diminished ribosome binding because of steric hinderance. Consequently, the inhibitory effect of SRP on ppAUT translation may be to reduce the number of ribosomes able to initiate protein synthesis, thereby lowering the level of completed 64-mer polypeptide at later time points. Hence, ppAUT release after 30 min (Fig. 2, panels E and F) probably resulted from polypeptide chains initiated at early times.

The importance of SRP-mediated elongation arrest for efficient protein translocation has not been definitively established. Elongation arrest is only elicited if translation is performed in the presence of SRP and the absence of membranes, reaction conditions that do not occur in intact cells. Furthermore, observing elongation arrest is highly dependent upon the particular mRNA being translated and the source of the in vitro translation system. Initial studies used preprolactin (Walter et al., 1981a) or pre-light chain (Meyer et al., 1982) as model proteins, and performed translations in the wheat germ cell-free system supplemented with canine SRP. Under these conditions, elongation arrest was essentially permanent in the absence of membranes. Yet our results corroborate those of Lipp et al. (1987) and demonstrate that elongation arrest for certain polypeptides was transient even in the wheat germ system. When preprolactin mRNA was translated in a rabbit reticulocyte lysate supplemented with canine SRP, elongation arrest was only detected as a transient (1–2 min) delay in the ribosomal transit time during synchronized translations (Wolin and Walter, 1989). Similarly, wheat germ SRP added to a wheat germ cell-free system did not arrest translation of human pre-placental lactogen or of a plant secretory protein (Prehn et al., 1987). These results suggest that observations of permanent SRP inhibition result from using a translation system composed of heterologous components.

Our data suggest that at least in vitro, transient translation arrest does play an important role in enhancing translational efficiency. In a previous study (Okun et al., 1990), we observed that ppPUT and ppAUT translocated into membranes less efficiently than preproinsulin. These results can now be explained in light of our findings that reducing precursor size reduces SRP-mediated inhibition. Elongation arrest allows the SRP-ribosome-nascent chain complex suffi-

**Table I**

Estimation of the ribosomal transit times for preproinsulin and preproinsulin-derived polypeptides in the presence or absence of SRP

Preproinsulin and the respective truncated polypeptides were synthesized in a synchronized translation reaction in the presence or absence of SRP (see "Experimental Procedures"). At various times, aliquots of the translation products were removed and treated with 2% CTAB. The CTAB-precipitable polypeptides were analyzed by SDS-PAGE, and the fluorographs subjected to densitometric analysis. The "transit time" corresponds to the time at which the densitometric intensity of the completed protein reaches its maximum value. Δ transit time is defined as the transit time in the absence of SRP subtracted from the transit time in the presence of SRP.

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<th>Precursor</th>
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**Fig. 5. Time course of SRP-mediated inhibition of ppPLC-A CAT translation.** ppPLC-A CAT mRNA encoding a fusion protein of 313 residues consisting of the first 78 residues of preproinsulin plus a linker peptide fused to chloramphenicol acetyltransferase (Esskridge and Shields, 1986) and globin mRNA were translated in the absence (lanes 1–12) or presence (lanes 14–25) of SRP (720 units/ml). At 15-min intervals, aliquots of the translation products were removed and treated with 2% CTAB. The CTAB-precipitable polypeptides (lanes 1–6 and 14–19) and soluble material (lanes 7–12 and 20–25) were analyzed by SDS-PAGE. Lane 13, 14<sup>C</sup> molecular weight markers from top to bottom are: 43, 25.7, 18.4, 14.3, 6.2, and 3.4 kDa, respectively. The arrows indicate the migration of 78-CAT (ppPLC-A CAT, M<sub>r</sub> ~27,000) and globin, respectively.
cient time to target to the SRP receptor prior to protein termination. Decreasing protein size increases the likelihood that translation terminates prior to membrane targeting, thereby decreasing translocation efficiency. By expressing ppPUT and ppAUT in cells and determining if in vivo translocation of these proteins is inefficient relative to prepri- 

sulin, it may be possible to prove that efficient translocation depends on elongation arrest. It is noteworthy that there are very few natural preproteins smaller than 70–80 amino acids and that translocation efficiency in vivo is virtually 100%. In this context, recent experiments (Schlenstedt et al., 1990) demonstrated that preprocecropin, 64 amino acids, can be translocated across the ER membrane by both co- and posttranslational mechanisms in a rabbit reticulocyte lysate cell-free system. Post-translational insertion was independent of ribosomes, SRP, and SRP-receptor but required ATP and an ATP-binding protein in the ER membrane (Klappa et al., 1991). However, the post-translational translocation of preprocecropin was significantly less efficient (approximately 10-

fold) than its co-translational insertion in the presence of SRP (Schlenstedt et al., 1990). In contrast to preprocecropin, our previous data (Okun et al., 1990) showed that neither native preprocecropin nor the various truncations were capable of post-translational insertion in the wheat germ system. In light of the observation that synthetic preprocecropin required prior denaturation in dimethyl sulfoxide to affect post-translational insertion (Klappa et al., 1991), it would be of interest to partially denature the truncated preprocecropin molecules and determine if these molecules could then support posttranslational membrane insertion.

SRP cannot induce elongation arrest at every position within the SRP window. CTAB precipitates of the in vitro translation reactions programmed with pPiPLC AT mRNA (313 amino acid) and performed in the presence of SRP revealed that the largest nascent chain size class is ~17 kDa. This value is quite close to that observed by Lipp et al. (1987), who reported a maximum size for granulocyte-macrophage colony-stimulating factor of 18 kDa, and a maximum size for I-γ of ~23 kDa. I-γ is a type II membrane protein that contains 30 hydrophilic amino acids NH₂-terminal to an internal signal sequence (Lipp and Dobberstein, 1986). After accounting for this 30-amino acid sequence, the distance between the signal sequence and the ribosomal A site at the limit of SRP binding is ~18 kDa. We speculate that this conserved value for several proteins may reflect a fundamental geometrical constraint in SRP, as it must bind both signal sequence and ribosome simultaneously to trigger elongation arrest.

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