

Signal Recognition Particle Mediates a Transient Elongation Arrest of Preprolactin in Reticulocyte Lysate

Sandra L. Wolin and Peter Walter

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

Abstract. Signal recognition particle (SRP) is a ribonucleoprotein that functions in the targeting of ribosomes synthesizing presecretory proteins to the ER. SRP binds to the signal sequence as it emerges from the ribosome, and in wheat germ extracts, arrests further elongation. The translation arrest is released when SRP interacts with its receptor on the ER membrane. We show that the delay of elongation mediated by SRP is not unique to wheat germ translation ex-

tracts. Addition of mammalian SRP to reticulocyte lysates resulted in a delay of preprolactin synthesis due to increased ribosome pausing at specific sites on preprolactin mRNA. Addition of canine pancreatic microsomal membranes to reticulocyte lysates resulted in an acceleration of preprolactin synthesis, suggesting that the endogenous SRP present in the reticulocyte lysate also delays synthesis of secretory proteins.

SIGNAL recognition particle (SRP)¹ is an 11S ribonucleoprotein particle that facilitates the translocation of nascent secretory proteins across the rough endoplasmic reticulum. The availability of extracts that reproduce secretory protein translocation has made it possible to derive a model for SRP function. According to this model, SRP binds with a low but measurable affinity to all ribosomes. Upon emergence of the signal sequence of a newly translated protein from the ribosome, the affinity of SRP for the ribosome increases by several orders of magnitude. The ribosome-nascent chain-SRP complex is then targeted to the endoplasmic reticulum, where SRP interacts with the SRP receptor (also called docking protein). SRP can thus be thought of as an adapter between the cytoplasmic protein synthetic machinery and the translocation apparatus in the endoplasmic reticulum membrane (reviewed by Walter and Lingappa, 1986; Hortsch and Meyer, 1986).

Many of the experiments leading to the above model were performed in a wheat germ translation extract that was supplemented with canine SRP and canine rough microsomes. If SRP is added to these extracts in the absence of microsomal membranes, SRP arrests or retards translation of the nascent polypeptide chain (Walter and Blobel, 1981; Ibrahim, 1987; Rapoport et al., 1987). This "elongation arrest" occurs after the nascent chain is at least 70 amino acids long. As 30-40 amino acids of the growing polypeptide chain are sequestered within the ribosome, this corresponds to the length at which the signal sequence has emerged completely from the ribosome. For certain proteins, a discrete "arrested fragment" can be detected (Walter and Blobel, 1981; Meyer

et al., 1982), while for other proteins, SRP arrest occurs at multiple sites during peptide elongation (Lipp et al., 1987).

An SRP-mediated delay in the synthesis of secretory proteins could serve at least two distinct functions. First, a pause in polypeptide elongation would increase the length of time that the nascent polypeptide remains in an unfolded conformation compatible with efficient translocation. Second, an elongation arrest could provide a regulatory mechanism by which cells rapidly modulate the synthesis of secretory proteins in response to environmental changes.

Because the ability of SRP to delay the translation of secretory proteins has important functional implications, it is necessary to demonstrate that this phenomenon is not unique to a wheat germ translation system supplemented with canine SRP. Several attempts to demonstrate an SRP-mediated translation arrest in other systems have been unsuccessful. Meyer (1985) found that the addition of even very high amounts of canine SRP had no effect on the synthesis of a secretory protein in either rabbit reticulocyte or HeLa cell lysates. Also, fractions containing either maize or wheat germ SRP did not arrest translation when added to wheat germ extracts (Prehn et al., 1987; Campos et al., 1988). Thus, the ability of SRP to arrest translation has been called into question as an artifact of an *in vitro* system reconstituted from heterologous components.

We have reinvestigated the effects of SRP on translation of secretory proteins in the rabbit reticulocyte lysate. We demonstrate that the addition of even low amounts of canine SRP causes a measurable delay in polypeptide elongation. Our results also indicate that the endogenous SRP present in the reticulocyte lysate specifically slows elongation of nascent secretory proteins.

1. *Abbreviations used in this paper:* 7mG, 7-methylguanosine-5'-monophosphate; SRP, signal recognition particle.

Materials and Methods

Reagents

Reticulocyte lysate was prepared as described by Jackson and Hunt (1983). SRP was prepared as described by Walter and Blobel (1983b), frozen in liquid nitrogen after the DEAE-Sephadex step, and stored at -80°C . Canine microsomal membranes were prepared according to Walter and Blobel (1983a), except that the column-washing step was omitted. The membranes were salt washed by adding an equal volume of 1 M potassium acetate, 50 mM triethanolamine-acetic acid, pH 7.5, (TEA), 50 mM EDTA, 1 mM DTT, and pelleting through a cushion of 500 mM sucrose, 50 mM TEA, 1 mM DTT. After two washes, the membranes were resuspended in the original volume of 500 mM sucrose, 50 mM TEA, 1 mM DTT. T4 DNA polymerase, gene 45 and genes 44/62 proteins were obtained as gifts from Drs. Jack Barry and Bruce Alberts (University of California, San Francisco).

In Vitro Transcription

Transcription of the plasmid pSPBP4, encoding bovine preprolactin, was as described previously (Wolin and Walter, 1988). For transcription of a truncated *Xenopus* lamin L₁ protein, the 1761 nucleotide Eco RI fragment of cDNA clone λ 3/700 (Krohne et al., 1987) was subcloned into the Eco RI site of pGEM2. The resulting plasmid was linearized with Sal I, and transcribed with SP6 polymerase as described (Wolin and Walter, 1988). The plasmid CYC Δ 90 encoding sea urchin B cyclin was a kind gift of Dr. Andrew Murray. In this construction (Murray et al., 1989), pSPBP4 was used as the parent plasmid, with the result that the transcribed cyclin and preprolactin mRNAs have identical 5' untranslated regions.

Synchronized Translations

A translation cocktail was prepared containing all components required for translation. Aliquots of the cocktail were added to tubes containing SRP, membranes, or buffer. The ionic conditions of the translations were maintained at 7 mM Tris, pH 7.5, 150 mM potassium acetate, 2 mM magnesium acetate, 0.6 mM DTT. The tubes were incubated at 26°C for 2 min to allow initiation to occur, at which time all further initiation was blocked by the addition of 7-methylguanosine-5'-monophosphate (7mG) to a final concentration of 10 mM. At various times thereafter, aliquots were removed and added to tubes containing SDS sample buffer (83 mM Tris base, 30% glycerol, 6.8% SDS, 240 mM DTT). The samples were heated to 100°C for 5 min, and then fractionated in a 10–15% polyacrylamide SDS gel. After autoradiography, bands were quantitated by scanning densitometry. As reticulocyte lysate translations are frequently carried out at 30°C (Jackson and Hunt, 1983), we also examined the effects of adding 30 nM SRP to translations performed at this temperature. At 30°C , we observed an SRP-mediated delay in preprolactin elongation identical to that seen at 26°C (data not shown).

Mapping of Ribosome-protected Fragments

Isolation of ribosome-protected fragments and mapping of their positions on the mRNA was performed as previously described (Wolin and Walter, 1988), except that additional CaCl_2 was added during the micrococcal nuclease digestion step to give a final calcium concentration of 3 mM.

Results

Exogenous SRP Delays Elongation of Nascent Preprolactin in Reticulocyte Lysate

To determine if added canine SRP affects the translation of secretory proteins in reticulocyte lysate, we measured the time required for the synthesis of full-length bovine preprolactin. We synchronized a translation reaction by allowing initiation to occur for 2 min, at which time further initiation was inhibited by the addition of the cap analogue 7mG to 10 mM. Aliquots of the reaction were removed at various times thereafter and analyzed by SDS-PAGE. In the absence of added canine SRP, completed preprolactin chains were first

observed after 10 min of synthesis (Fig. 1 A). In the presence of 200 nM canine SRP, however, full-length preprolactin did not appear until 12–13 min of synthesis (Fig. 1 B). As an internal control, we monitored the translation of a nonsecretory protein, *Xenopus* lamin L₁ (Krohne et al., 1987). Synthesis of this control protein was unaffected by even this high SRP concentration (compare Fig. 1, A with B), demonstrating that the delay in preprolactin synthesis was not due to a slowing of overall protein synthesis.

We next examined the effects of adding lower amounts of canine SRP to the reticulocyte lysate. (Most mammalian cells contain 10–20 nM SRP.) As shown in Fig. 1 C, addition of 100 nM SRP resulted in a 2-min delay in the appearance of full-length preprolactin. Similarly, addition of 30 nM SRP resulted in a 1-min delay of synthesis. As an internal control in these experiments, we examined the translation of another non-secretory protein, sea urchin cyclin (Pines and Hunt, 1987; Murray et al., 1989). Again, as shown in Fig. 1 D, the translation of this control protein was unaffected by the addition of SRP. These results demonstrate that exogenous SRP specifically retards the synthesis of a secretory protein in the reticulocyte lysate.

SRP Enhances Ribosome Pausing at Specific Sites on Preprolactin mRNA

To determine the regions of the mRNA at which SRP was slowing translation of preprolactin, we used a recently developed assay for monitoring ribosome movement along an mRNA (Wolin and Walter, 1988). In this assay, translation extracts are treated with micrococcal nuclease and the mRNA fragments protected from digestion by translating ribosomes are purified. The positions of stalled ribosomes on the mRNA are then mapped with a primer extension assay. The ribosome-protected fragments are hybridized to the antisense cDNA strand of the mRNA of interest. A 5'-labeled oligonucleotide primer is annealed upstream of the protected fragments and extended with T4 DNA polymerase. Because this polymerase will not displace the hybridized mRNA fragment, the enzyme stops at the 5' end of the ribosome-protected fragment. The positions of the stalled ribosomes can then be determined with single nucleotide precision by using the same labeled oligonucleotide in a dideoxy sequencing reaction. The gel bands generated in this assay thus correspond to the trailing edges of the stalled ribosomes.

During translation of preprolactin mRNA in both wheat germ extracts and reticulocyte lysates, four major positions of ribosome stalling are detected (Wolin and Walter, 1988; also see Fig. 2 A, lanes 1 and 4). Two of these positions of ribosome pausing, designated *a* and *d* in Fig. 2, correspond to initiation and termination of translation, respectively (Wolin and Walter, 1988). There are also two internal positions of ribosome stalling. One of these positions, designated *b* in Fig. 2, corresponds to nascent polypeptide chains of 63–77 amino acids. The other internal phase (*c*) corresponds to between 131 and 165 amino acids. Note that for pauses *b*, *c*, and *d*, each position of ribosome stalling is represented by a group of closely spaced bands. The slowest migrating band in each group represents pausing by the leading ribosome; the other prominent bands are derived from the stacking of upstream ribosomes behind the paused ribosome (Wolin and Walter, 1988).

Using this assay, we examined the effects of added SRP on

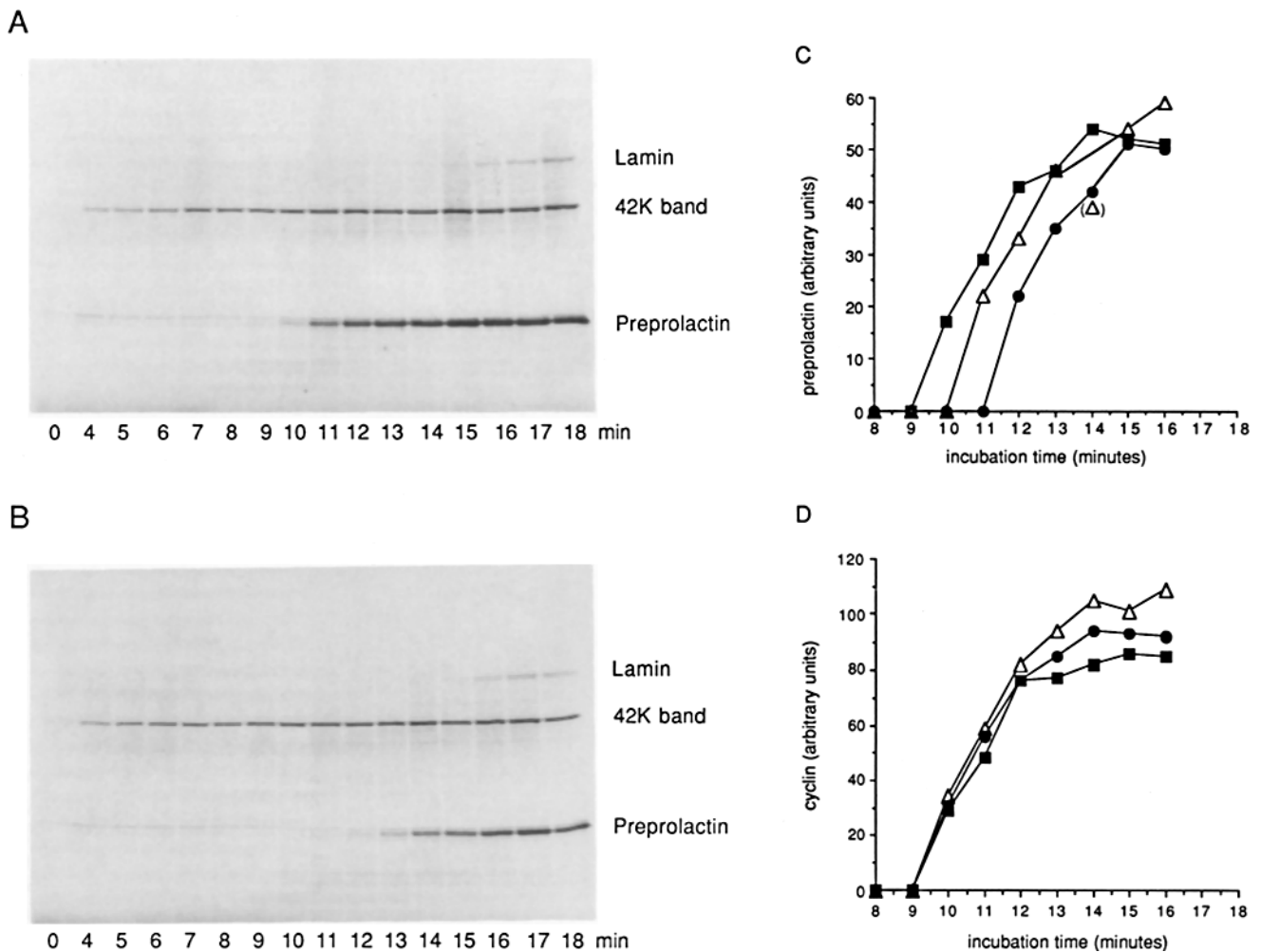


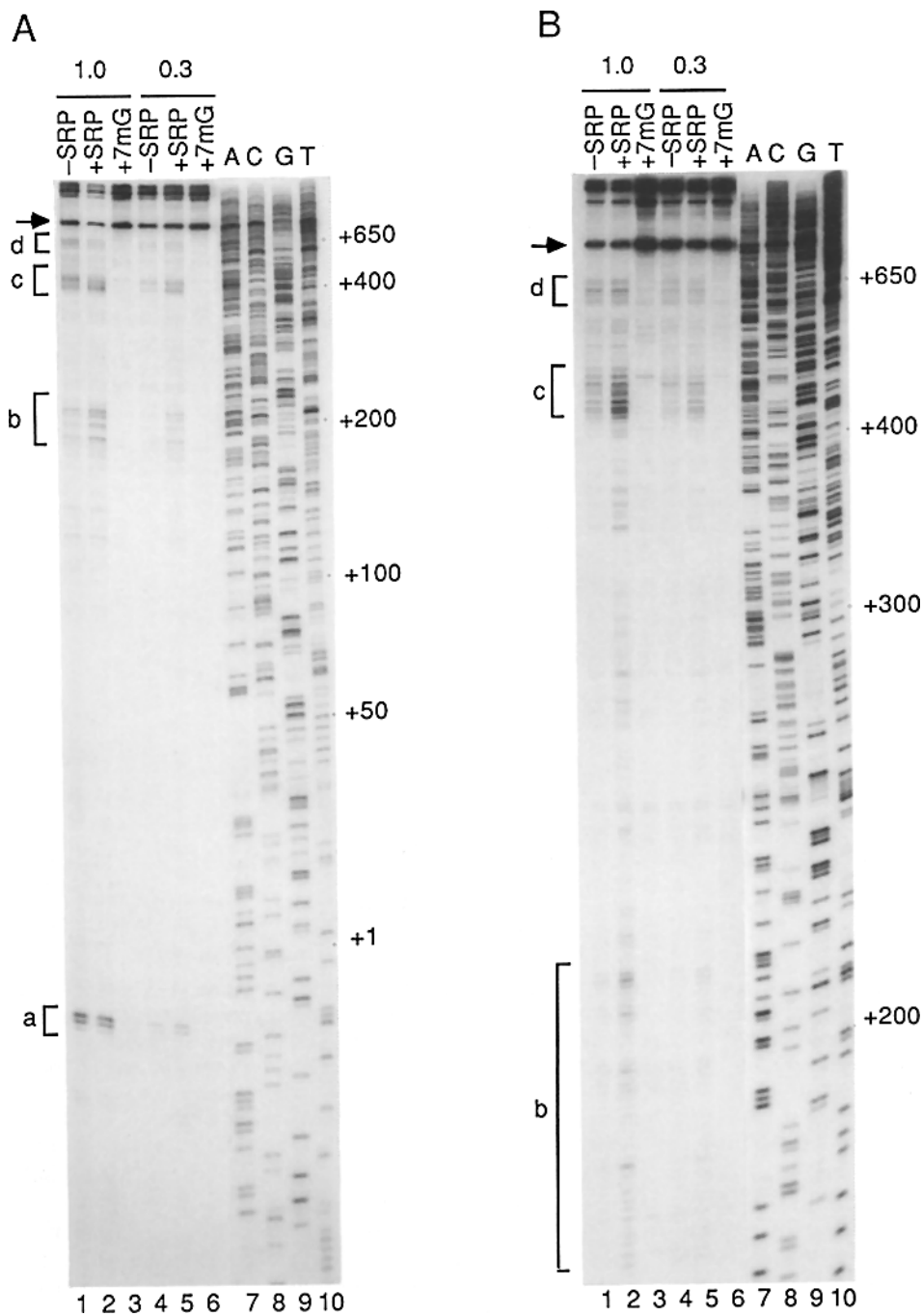
Figure 1. Addition of canine SRP delays synthesis of preprolactin in reticulocyte lysates. (A and B) Synthetic mRNAs encoding either bovine preprolactin or *Xenopus* lamin L₁ were translated in a synchronized rabbit reticulocyte lysate as described in Materials and Methods. The translation reaction contained either (A) no added canine SRP or (B) 200 nM SRP. Aliquots were sampled at the times shown and analyzed by SDS-PAGE. The band labeled 42K is due to posttranslational addition of methionine to an endogenous lysate protein (Jackson and Hunt, 1983). (C and D) Synthetic mRNAs encoding either bovine preprolactin (C) or sea urchin cyclin (D) were translated in a synchronized rabbit reticulocyte lysate as described in Materials and Methods. Translation reactions contained either no added SRP (■), 30 nM SRP (●), or 100 nM SRP (Δ). Aliquots were sampled at the times indicated and analyzed by SDS-PAGE and scanning densitometry as described.

ribosome pausing in the reticulocyte lysate. When 200 nM SRP was added to reticulocyte lysates, ribosome pausing at the two internal positions, *b* and *c*, was enhanced (Fig. 2, A and B, compare lanes 1 and 4 with lanes 2 and 5). In contrast, ribosome stalling at positions *a* and *d* (initiation and termination) appears unaffected by SRP addition. Thus, enhanced ribosome stalling caused by SRP at the two internal pause sites on preprolactin mRNA is consistent with the known ability of SRP to delay the synthesis of nascent secretory proteins at discrete points. (The enhancements of minor bands, notably between pause regions *b* and *c* [Fig. 2 B, compare lanes 1 and 2] may be due either to increased ribosome pausing at these sites or to increased ribosome stacking behind ribosomes stalled at a major pause region.)

The Endogenous SRP in Reticulocyte Lysates Slows Elongation of Preprolactin

It was previously shown that reticulocyte lysates contain en-

dogenous SRP (Meyer et al., 1982). By quantitating the amount of SRP RNA in our reticulocyte lysate by ethidium bromide staining, we have determined that our translation reactions contain ~5 nM SRP (data not shown). To determine if this endogenous SRP causes a transient pause during preprolactin elongation, we added canine microsomal membranes (salt-washed to remove SRP) to the translation reaction. These membranes, which contain SRP receptor, should relieve any transient arrest of translation mediated by the endogenous reticulocyte SRP. However, microsomal membranes also cause a nonspecific inhibition of overall protein synthesis (Walter and Blobel, 1983). In the presence of microsomal membranes, the appearance of the control non-secretory protein, sea urchin cyclin, was delayed by 1 min, and the total amount of cyclin made at the end of the time course was reduced by 50% (Fig. 3, compare A with B; also see Fig. 3 D). In contrast, the addition of microsomal membranes actually speeded up the synthesis of prolactin by



protected fragments are present (Fig. 2 *A*, compare lanes 2 and 3), many of the polymerase molecules stop before encountering a strong stop in the M13 vector (arrow), and the signal corresponding to ribosomes pausing at pause region *d* appears artifactually low. When the amount of ribosome protected fragments in the reaction is reduced (Fig. 2 *A*, lanes 4–6), the vast majority of polymerase molecules traverse the entire cDNA, indicating that the hybridization reaction was performed under conditions of DNA excess. The minor difference in pause region *a* and lanes 4 and 5 of *A* is not reproducible.

1 min. Note that membranes were not added in saturating amounts and that the portion of nontranslocated preprolactin synthesized appeared with a time course identical to preprolactin synthesized in the absence of membranes (arrow in Fig. 3 *B*; also Fig. 3 *C*). Thus, it appears that the endogenous SRP present in the reticulocyte lysate slows ribosomes engaged in the synthesis of preprolactin, and this transient pausing is relieved by the addition of microsomal membranes containing SRP receptor.

Discussion

We have shown that an SRP-mediated delay of secretory protein synthesis, previously demonstrated to occur in wheat germ translation extracts supplemented with canine SRP, also occurs in reticulocyte lysates containing endogenous SRP. By using synchronized reticulocyte lysates to monitor the time required to synthesize full-length proteins, we have demonstrated that added canine SRP delays elongation of a

Figure 2. Added canine SRP mediates pausing of reticulocyte lysate ribosomes at specific sites during translation of preprolactin mRNA. Total ribosome-protected fragments were isolated from reticulocyte lysate translations containing either no SRP (lanes 1 and 4), 200 nM SRP (lanes 2 and 5), or 10 nM 7mG (lanes 3 and 6) as described (Wolin and Walter, 1988). Ribosome-protected fragments were analyzed by annealing to the anti-sense cDNA strand of the mRNA. A 5'-labeled oligonucleotide primer was also annealed to the cDNA clone. In *A*, the primer consisted of the M13-40 primer (GTTTCCCAGTCCGAC), and in *B*, the primer consisted of the oligonucleotide GGTATCCCTTCGAGACC, which spans nucleotides 126–142 of the preprolactin coding sequence. The positions of the fragments were mapped by extending the labeled primer with T4 DNA polymerase and the genes 44/62 and gene 45 accessory proteins. The primer extension products were fractionated in a 8.3 M urea, 5% polyacrylamide gel. To generate molecular size markers, the labeled oligonucleotides were also used in dideoxy sequencing reactions with reverse transcriptase. As described previously (Wolin and Walter, 1988), if two ribosome-protected fragments anneal to the same molecule of DNA, the polymerase will stop upon encountering the first fragment, and the signal corresponding to the downstream fragment will be lost. We therefore varied the amount of RNA fragments, relative to a constant amount of DNA, in the hybridization reaction. Numbers at the top represent microliter amounts of ribosome-protected fragments used in each reaction. Thus, when high amounts of pro-

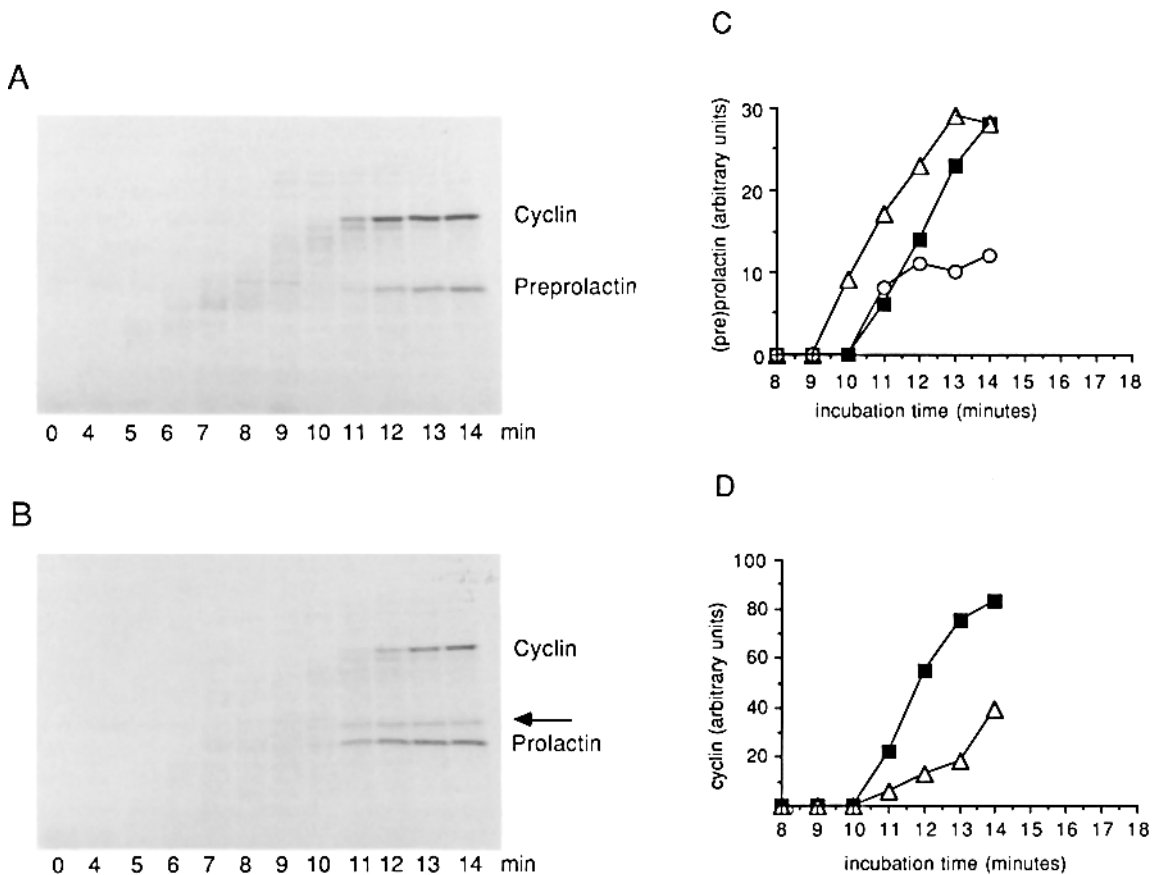


Figure 3. Addition of canine microsomal membranes accelerates elongation of preprolactin in reticulocyte lysates. (A and B) Synthetic mRNAs encoding either bovine preprolactin or sea urchin cyclin were translated in a synchronized rabbit reticulocyte lysate as described in Materials and Methods. Translation reactions (80 μ l) contained either (A) no added membranes or (B) 2 μ l microsomal membranes. Aliquots (5 μ l) were removed at the times indicated and analyzed by SDS-PAGE followed by autoradiography. The arrow in B designates residual preprolactin synthesis. (C and D) The synthesis of bovine preprolactin and prolactin (C) and sea urchin cyclin (D) was quantitated by scanning densitometry of the autoradiograms shown in A and B. ■, - microsomal membranes; Δ , + microsomal membranes; O, residual preprolactin synthesis in presence of microsomal membranes. Note that although the addition of membranes inhibits the synthesis of cyclin by 50%, the total amount of prolactin made is unaffected. This is probably because the effect of releasing the arrest of preprolactin mediated by the endogenous SRP is larger in magnitude than the nonspecific slowing of overall protein synthesis due to addition of microsomal membranes.

nascent secretory protein, bovine preprolactin. This delay is associated with ribosome pausing at specific sites on preprolactin mRNA after the signal sequence has completely emerged from the ribosome. Addition of microsomal membranes resulted in an increase in the rate of preprolactin synthesis, strongly suggesting that the endogenous SRP present in reticulocyte lysates also specifically delays the elongation of nascent secretory proteins.

An SRP dependent delay of elongation of two secretory proteins, preprolactin and a fusion protein containing the signal peptide of *E. coli* outer membrane lipoprotein fused to β -lactamase (Garcia et al., 1987), has also been observed in a translation extract reconstituted from mammalian components (Matlack, K., and P. Walter, unpublished data). Although Meyer (1985) failed to detect any effect of canine SRP on translation in either a reticulocyte lysate or a HeLa cell extract, this may be because only steady-state accumulation of secretory proteins, rather than time required for synthesis, was measured. The failure of others (Shields and Blobel, 1978; Meyer et al., 1982) to detect increased secretory protein synthesis upon addition of microsomal membranes to

reticulocyte lysates is probably also due to the fact that only steady-state synthesis was assayed.

While the general phenomenon of SRP-mediated ribosome pausing occurs in both reticulocyte lysates and wheat germ extracts, the degree of pausing varies considerably between the two translation systems. Although in both cases SRP must bind to the nascent chain-ribosome complex to promote translocation, subtle changes in the translation machinery between the two systems may affect the extent to which polypeptide elongation is retarded. The precise point in the elongation cycle at which SRP blocks translation has not yet been defined. It is possible that variations in either the level or affinity of an elongation factor or the level of uncharged tRNA affect the ability of SRP to halt elongation. Alternatively, the more pronounced effect of canine SRP on translation in the wheat germ system may result from the heterologous nature of the components.

The overall magnitude of the described effects in the mammalian translation system, compared to the wheat germ system, is small. However, even a one minute delay in protein elongation could have drastic consequences in vivo and thus

be of functional significance. Although an arrest of translation is not required for translation in vitro (Siegel and Walter, 1985), a delay in polypeptide elongation would increase the length of time the nascent polypeptide chain remains in a translocation-competent state. Also, if changes in the translation apparatus affect the extent to which SRP retards polypeptide elongation, an SRP-mediated delay of ribosome transit could modulate the level of secretory protein synthesis in response to an extracellular stimulus.

We thank Kent Matlack for his contributions to the early stages of this work and for allowing us to cite his unpublished data. We are grateful to Bruce Alberts and Jack Barry for providing T4 DNA polymerase and accessory proteins and to Andrew Murray for providing pCYCΔ90. Carl Hashimoto, Vivian Siegel, and Katharina Strub made helpful comments on the manuscript.

S. L. Wolin is a Lucille P. Markey Scholar and this work was supported in part by a grant from the Lucille P. Markey Charitable Trust. This work was also supported by grants from the National Institutes of Health (GM-32384) and the Juvenile Diabetes Foundation (187668).

Received for publication 21 June 1989 and in revised form 2 August 1989.

References

Campos, N., J. Palau, M. Torrent, and D. Ludevid. 1988. Signal recognition-like particles are present in maize. *J. Biol. Chem.* 263:9646-9650.

Garcia, P. D., J. Ghayeb, M. Inouye, and P. Walter. 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.

Hortsch, M., and D. I. Meyer. 1986. Transfer of secretory proteins through the membrane of the endoplasmic reticulum. *Int. Rev. Cytol.* 102:215-242.

Ibrahimi, I. 1987. Signal recognition particle causes a transient arrest in the biosynthesis of preprolactin and mediates its translocation across mammalian endoplasmic reticulum. *J. Cell Biol.* 104:61-66.

Jackson, R. J., and T. Hunt. 1983. Preparation and use of nuclease-treated rabbit reticulocyte lysates for the translation of eukaryotic messenger RNA. *Methods Enzymol.* 96:50-74.

Krohne, G., S. L. Wolin, F. D. McKeon, W. W. Franke, and M. W. Kirschner. 1987. Nuclear lamin L₁ of *Xenopus laevis*: cDNA cloning, amino acid se-

quence and binding specificity of a member of the lamin B subfamily. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3801-3808.

Lipp, J., B. Dobberstein, and M.-T. Haepfle. 1987. Signal recognition particle arrests elongation of nascent secretory and membrane proteins at multiple sites in a transient manner. *J. Biol. Chem.* 262:1680-1684.

Meyer, D. I. 1985. Signal recognition particle (SRP) does not mediate a translational arrest of nascent secretory proteins in mammalian cell-free systems. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:2031-2033.

Meyer, D. I., E. Krause, and B. Dobberstein. 1982. Secretory protein translocation across membranes—the role of the “docking protein.” *Nature (Lond.)* 297:647-650.

Murray, A. W., M. J. Solomon, and M. W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature (Lond.)* 339:280-285.

Prehn, S., M. Wiedmann, T. A. Rapoport, and C. Zwieb. 1987. Protein translocation across wheat germ microsomal membranes requires an SRP-like component. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2093-2097.

Pines, J., and T. Hunt. 1987. Molecular cloning and characterization of the mRNA for cyclin from sea urchin eggs. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:2987-2995.

Rapoport, T. A., R. Heinrich, P. Walter, and T. Schulmeister. 1987. Mathematical modeling of the effects of signal recognition particle on translation and translocation of proteins across the endoplasmic reticulum membrane. *J. Mol. Biol.* 195:621-636.

Shields, D., and G. Blobel. 1978. Efficient cleavage and segregation of nascent presecretory proteins in a reticulocyte lysate supplemented with microsomal membranes. *J. Biol. Chem.* 253:3753-3756.

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

Siegel, V., and P. Walter. 1988. The affinity of signal recognition particle for presecretory proteins is dependent on nascent chain length. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1769-1775.

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

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 V. R. Lingappa. 1986. Mechanism of protein translocation across endoplasmic reticulum membrane. *Annu. Rev. Cell Biol.* 2:499-516.

Wolin, S. L., and P. Walter. 1988. Ribosome pausing and stacking during translation of a eukaryotic mRNA. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3559-3569.