Interactions of signal peptides with signal-recognition particle

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The mechanisms whereby isolated or synthetic signal peptides inhibit processing of newly synthesized prolactin in microsome-supplemented lysates from reticulocytes and wheat-germ were investigated. At a concentration of 5 μ M, a consensus signal peptide reverses the elongation arrest imposed by the signal-recognition particle (SRP), and at higher concentrations in addition inhibits elongation of both secretory and non-secretory proteins. A photoreactive form of a synthetic signal peptide cross-links under u.v. illumination to the 54 kDa and 68 kDa subunits of SRP, whereas the major cross-linked protein produced after photoreaction of rough microsomes is of 45 kDa. As SRP-mediated elongation arrest is unlikely to be essential for translocation, it is suggested that signal peptides may interact with components other than SRP in the translation system *in vitro*.

INTRODUCTION

Newly synthesized secretory proteins are thought to be targeted to the membrane of the rough endoplasmic reticulum (RER) in mammalian cells by an 11 S ribonucleoprotein, signal-recognition particle (SRP) (Walter & Blobel, 1982). The signal sequence in a nascent chain as it emerges from the ribosome is thought to promote tight binding of the ribosomal complex to SRP, which is not released until contact is made with a specific receptor, docking protein, in the membrane of the RER (Walter & Blobel, 1981a; Meyer *et al.*, 1982). Originally isolated from salt extracts of pancreatic rough microsomes, SRP has been shown to reconstitute *in vitro* the ability to translocate and process newly synthesized secretory and plasma-membrane pre-proteins to SRP-depleted salt-extracted rough microsomes (Walter & Blobel, 1980; Anderson *et al.*, 1982; Bassuner *et al.*, 1984).

SRP halts elongation of preprolactin in a synchronized wheat-germ translation system to yield an incomplete chain of 8 kDa (70 residues in length) (Walter & Blobel, 1981b), and the point of elongation arrest coincides with rare codon usage on the mRNA (Wolin & Walter, 1988). In cell-free translation systems prepared from mammalian tissue, SRP does not exert the complete elongation arrest observed in wheat-germ lysates (Meyer, 1985). SRP has been shown to retain the alpha-mating factor precursor in a translocation-competent conformation, i.e. a state which allows it to cross the membrane post-translationally in a yeast cell-free system (Sanz & Meyer, 1988). However, the ways in which signal sequences in newly synthesized proteins mediate the various functions of SRP are not completely understood.

We have shown previously that synthetic or isolated signal peptides inhibit the translocation and processing of newly synthesized secretory pre-proteins in a rabbit reticulocyte translation system (Austen *et al.*, 1984; Robinson *et al.*, 1986). The inhibition of translocation by signal peptides could be due to interference with the normal engagement that SRP makes with polysomes translating mRNA, or with subsequent functional interactions that signal peptides make with components of the

ER membrane as passage of the newly synthesized polypeptide chain across the phospholipid bilayer takes place. In order to understand more fully how signal sequences in pre-proteins function, we have isolated SRP, examined some of its properties, and explored the effects that signal peptides have on SRP-mediated processing and elongation arrest *in vitro*.

MATERIALS AND METHODS

Materials

All biochemical reagents were obtained from Sigma or BDH, of Poole, Dorset, U.K., and were of the purest grade available.

Isolation of SRP and salt-extracted microsomes

Gel-filtered dog pancreatic rough microsomes were isolated as described by Kaderbhai & Austen (1984) and diluted to a 20 ml suspension of 50 A_{280} units/ml with 0.25 M-sucrose/20 mM-Hepes/KOH (pH 7.4). SRP was purified by a modification of the method of Walter & Blobel (1980). Ice-cold 1.5 M-potassium acetate/15 mM-magnesium acetate (pH 7.5) (10 ml) was added to the microsomes, and they were vortex-mixed briefly, left on ice for 15 min and centrifuged through a cushion (10 ml) of 0.5 M-sucrose/50 mM-triethanolamine acetate (pH 7.5)/0.5 M-potassium acetate/5 mM-magnesium acetate/1 mM-dithiothreitol at 140 000 g_{av} for 1 h at 4 °C. The supernatant was centrifuged at 140 000 g_{av} for a further 3 h at 4 °C to deplete it of ribosomes, and the post-ribosomal supernatant was passed over a column (1 cm $\times$ 3 cm) of ω -aminopentyl-agarose at 4 °C, and washed through with 0.5 M-potassium acetate/5 mM-magnesium acetate/1 mM-dithiothreitol/50 mM-triethanolamine acetate (pH 7.5) at 3 ml/h. Bound SRP was eluted with a 10 ml step of 1 M-potassium acetate/10 mM-magnesium acetate/1 mM-dithiothreitol/50 mM-triethanolamine acetate (pH 7.5) containing 0.05% (w/v) Nikkol (Nikko Chemical Co., Tokyo, Japan), and collected as 0.75 ml fractions.

SRP isolated by affinity chromatography was located

Abbreviations used: SRP, signal-recognition particle; 2D-PAGE, two-dimensional separation on PAGE; RER, rough endoplasmic reticulum; PTH, parathyroid hormone; Ov, tryptic fragment bearing the signal sequence of ovalbumin.

in fractions by SDS/PAGE (see below), and protein concentration was measured by reaction with Coomassie Blue (Robinson, 1987). A small amount of the peak fraction (0.75 μ g of protein in 0.015 ml) was incubated with 125 I-labelled Bolton–Hunter reagent (37 μ Ci; 2000 Ci/mmol; Amersham International, Little Chalfont, Bucks., U.K.) for 3 h on ice, the reaction being terminated by addition of 0.185 ml of 54 mM-Tris/acetate (pH 7.4). Salt-extracted microsomes left after extraction of SRP were resuspended in 0.5 M-potassium acetate/5 mM-magnesium acetate, and washed by centrifugation once again through an 0.5 M-sucrose cushion, and the pellet was resuspended in 0.25 M-sucrose/20 mM-Hepes/KOH (pH 7.5) to a concentration of 50 A_{280} units/ml.

Translation *in vitro*

Nuclease-treated reticulocyte lysates were prepared and used as described by Jackson & Hunt (1983), and wheat-germ lysates as described by Erickson & Blobel (1983). Polyadenylated mRNA from bovine pituitary was added to translation mixtures at 2 A_{280} units/ml together with [35 S]methionine (5 mCi/ml). When solutions of SRP containing 1 M-potassium acetate and 10 mM-magnesium acetate were added, the concentrations of K^+ and Mg^{2+} of the translation cocktail (Erickson & Blobel, 1983) were decreased accordingly to maintain the optima for the mRNA translation, and Nikkol was added to control lysates so that its concentration was kept constant. Nuclease-treated or salt-stripped rough microsomes (Austen *et al.*, 1984) were added to 4 A_{280} units/ml when required. Synchronization of protein synthesis with respect to polypeptide-chain elongation was achieved by addition to the translation mixture of edeine (final concn. 10 μ M) a short time (3 min for wheat-germ lysate and 1 min for reticulocyte lysate) after protein synthesis had been initiated by addition of mRNA and warming the lysates to 25 °C (wheat-germ) or 30 °C (reticulocytes).

Polysome size analysis on sucrose gradients

Reticulocyte lysates translated for 15 min were diluted with 3 vol. of ice-cold 20% (w/v) sucrose in 20 mM-Tris/HCl (pH 7.4)/100 mM-KCl/5 mM-magnesium acetate, and centrifuged through 12 ml linear gradients of 20–50% sucrose in the same buffer at 40000 rev./min for 2.5 h in the Beckman SW41 rotor at 4 °C. A_{260} was monitored by pumping gradients through a flow-cell of a recording spectrophotometer into a fraction collector, and 35 S-labelled protein was measured after trichloroacetic acid precipitation of 10 μ l samples obtained from 0.5 ml fractions.

Gel electrophoresis

The protein subunits of SRP were separated on linear gradients of 10–15% polyacrylamide in SDS (Robinson *et al.*, 1987). 35 S-labelled translated proteins were separated by SDS/PAGE (15% acrylamide), and located by fluorography (Austen *et al.*, 1984). 125 I-labelled proteins were mixed with rough microsomal membranes (100 μ g of protein) and separated by 2D-PAGE (Kaderbhai & Austen, 1984) and localized by autoradiography (Robinson *et al.*, 1987).

Signal peptides

Synthesis and isolation of the consensus signal peptide (Austen *et al.*, 1984) and isolation of a tryptic fragment

Fig. 1. Isolation and PAGE of SRP

SRP was precipitated at 0 °C from 0.4 ml samples of column fractions by incubation first with 0.4 ml of 20% (v/v) trichloroacetic acid for 1 h, addition of 0.005 ml of 10% (w/v) sodium deoxycholate to act as a co-precipitant, and incubation for a further 10 min at 20 °C. After centrifugation at 12000 g_{av} for 15 min at 20 °C, the pellet was prepared for electrophoresis on gradients of 10–15% (w/v) acrylamide. The protein subunits of SRP are arrowed. Lanes a and i show high- and low-molecular-mass protein standards.

containing the signal sequence of ovalbumin (Robinson *et al.*, 1986) have been described. The synthetic fragment (residues 28–48) of parathyroid hormone (PTH) used as a control was purchased from Cambridge Research Biochemicals, Cambridge, U.K. Photolabelling of pancreatic rough microsomes and SRP with 125 I-labelled azidophenacyl-signal peptide was performed as described previously (Robinson *et al.*, 1987).

RESULTS

Isolation of SRP

The protein peak eluted by 1 M-potassium acetate and 0.05% Nikkol from a column of ω -aminopentyl-agarose exhibited on SDS/PAGE the six protein subunits previously described in SRP (molecular masses 72, 68, 54, 19, 14 and 9 kDa), the higher-molecular-mass subunits staining more intensely because of their increased mass (Fig. 1). The six subunits have previously been reported to exist in equimolar amounts (Walter & Blobel, 1980). In our preparation, contamination was low, and consisted of four minor protein bands, of 52, 51, 43 and 27 kDa.

Inhibition by signal peptides of translation and translocation of newly synthesized proteins

SRP has been reported to be present in reticulocyte lysates, but absent from wheat-germ lysates (Meyer, 1985). It was therefore of interest to compare the effects of signal peptides on translation and processing of newly synthesized preprolactin in the two systems.

In the absence of added nuclease-treated rough microsomes, marked inhibition of 35 S incorporation into protein was observed between 5 and 50 μ M concentrations

Fig. 2. Inhibition of translation by signal peptides

Shown is the radioactivity (c.p.m.) in ^{35}S -labelled protein precipitated with trichloroacetic acid from translations performed by addition of mRNA to reticulocyte lysates containing various concentrations of consensus signal peptide present throughout translation, except in one translation when edeine (final concn. $10\ \mu\text{M}$; + edeine) and the peptide, to the final concentrations shown, were added together 1 min after translation had been initiated by a temperature shift from 0°C to 30°C (Δ). Translations were either without nuclease-treated rough microsomes, with globin mRNA ($\blacktriangle$) or bovine pituitary mRNA (pPL) ($\square$), or plus microsomes at $4\ A_{280}$ units/ml (+ RER) with globin mRNA ($\bullet$) or pituitary mRNA ($\circ$). Protein synthesized in a wheat-germ lysate at 25°C from bovine pituitary mRNA in the presence of peptide is also shown ($\blacksquare$). The 100% value is the radioactivity (c.p.m.) acid-precipitated from each translation performed in the absence of peptide.

of consensus signal peptide (Fig. 2). The inhibitory effect was not specific for synthesis of secretory protein, as even greater inhibition was exerted on translation of globin mRNA. The peptide's inhibitory effect was alleviated by addition of microsomal membranes to a maximum of 15% (preprolactin) and 30% (globin) at $50\ \mu\text{M}$ concentration of peptide. To see if the peptide inhibited the initiation or elongation stage of protein synthesis, the re-initiation inhibitor edeine was added together with consensus signal peptide 1 min after translation had been initiated. Although the total trichloroacetic acid-precipitable radioactivity (c.p.m.) was decreased to one-fifth by the presence of edeine, the extent of inhibition by the peptide was the same as the inhibition detected in the absence of edeine, indicating that the peptide predominantly affected the elongation stage.

Globin mRNA was translated in reticulocyte lysates in both the presence and the absence of consensus signal peptide, and polysomes were then analysed by centrifugation on a sucrose gradient. Similar profiles of polysomes were obtained in each case, with 55% of ribosomes found as trimers or larger polysomes, indicating that the peptide has little inhibitory effect on initiation, which would have produced a build-up of monosomes. The peptide also inhibited translation of preprolactin in

Fig. 3. Inhibition of processing of preprolactin by signal peptide

Shown is the extent of processing of ^{35}S -preprolactin (pPL) to ^{35}S -prolactin (PL) translated from bovine pituitary mRNA in the presence of nuclease-treated microsomal membranes and various concentrations of the tryptic fragment bearing the signal sequence of ovalbumin in either a reticulocyte lysate ($\bullet$) or a wheat-germ lysate ($\square$). Processing was calculated from densitometry traces of fluorographs of the SDS/PAGE gels as described previously (Kaderbhai & Austen, 1984).

a wheat-germ system, but to a lesser extent than in the reticulocyte lysate. The ovalbumin signal fragment inhibited translation to similar extents in both types of lysates (results not shown).

The extent of co-translational processing of preprolactin was greater in the reticulocyte lysate than in the wheat-germ lysate, possibly because there is less SRP in the latter (Meyer, 1985) (Fig. 3). The tryptic fragment containing the signal region of ovalbumin was found to inhibit processing almost completely at concentrations between 2.5 and $25\ \mu\text{M}$ in both translation systems (Fig. 3). Pooled tryptic peptides isolated from a tryptic digest of ovalbumin, from which the tryptic fragment bearing the signal region had been removed by h.p.l.c. (Robinson *et al.*, 1986), inhibited translation, but did not affect the extent of processing achieved in the reticulocyte lysate.

A possible mode of interaction of signal peptides in the processing system *in vitro* could be interference with elongation arrest imposed by SRP. Firstly, the SRP isolated was tested for its reported activity in restoring translocation to salt-extracted microsomes in a processing system *in vitro*. Nuclease-treated pancreatic microsomes, which contain endogenous SRP, were found to give rise to 25% conversion of ^{35}S -preprolactin into ^{35}S -prolactin when present during translation in a wheat-germ translation system (Fig. 4, track 1). Conversion increased to 95% when extra isolated SRP ($5\ \mu\text{g}$ of protein) was added in addition (track 2). Extraction of rough microsomes by high salt depleted them of SRP, and the resulting salt-stripped microsomes converted only 10% of ^{35}S -preprolactin into its mature form (track 3). By adding back isolated SRP to the salt-extracted microsomes, the extent of conversion increased to 45% (track 4).

Fig. 4. SRP-dependence of processing and translocation

Shown is the fluorograph of ^{35}S -preprolactin (pPL) and ^{35}S -prolactin (PL) translated by the addition of bovine pituitary mRNA to a wheat-germ lysate translation mixture containing microsomes ($4 A_{280}$ units/ml) that were either nuclease-treated (RM_N) or salt-stripped (KM), with [^{35}S]methionine, and with or without added SRP ($5 \mu\text{g}$ of SRP protein/ml).

Having ascertained that our preparation of SRP was active, we investigated the actions of peptides on SRP-mediated elongation arrest. Translations were first synchronized shortly after protein synthesis had been initiated by addition of mRNA, by supplementing with the re-initiation inhibitor edeine (final concn. $10 \mu\text{M}$) 3 min after initiation. In a synchronized wheat-germ lysate, the amount of ^{35}S -preprolactin biosynthesized was unaffected by $0.1 \mu\text{M}$ consensus signal peptide (compare tracks 4 and 7 in Fig. 5a), but at $5 \mu\text{M}$ peptide the elongation arrest of SRP was reversed and 80% of the translation observed in the absence of SRP occurred. Reversal of elongation arrest was also apparent at $10 \mu\text{M}$ and $50 \mu\text{M}$ concentrations of peptide (compare tracks 9 and 10 with track 4), but at these higher concentrations less ^{35}S -preprolactin was visible on the fluorograph than with $5 \mu\text{M}$ peptide, presumably because, as described above, higher concentrations of peptide produce a more marked inhibitory effect on translation.

The isolated fragment (residues 21–47) containing the signal region of ovalbumin also reversed elongation arrest. To compensate for the peptide's inhibition of translation, incubations were performed in the absence and presence of SRP with equivalent concentrations of the ovalbumin peptide in one pair of translations, and a control hydrophobic synthetic peptide comprising residues 28–48 of parathyroid hormone (PTH) in another. The PTH peptide at $25 \mu\text{M}$ concentration had little effect

Fig. 5. Effect of consensus signal peptide on elongation arrest

[^{35}S]Methionine and bovine pituitary mRNA were added to a wheat-germ translation mixture containing various amounts of a stock solution of SRP ($50 \mu\text{g}$ of SRP protein/ml) added to give 2.5 – $8.0 \mu\text{g}$ of SRP protein/ml in the lysate and various concentrations of consensus signal peptide (CSP). At 3 min after a temperature shift from 0°C to 25°C , edeine was added (final concn. $10 \mu\text{M}$). Shown is the fluorograph of the translated preprolactin (pPL) (a), and radioactivity (c.p.m.) in ^{35}S -labelled protein precipitated by trichloroacetic acid (b) in the absence of peptide (■) and in the presence of $0.1 \mu\text{M}$ (□), $5 \mu\text{M}$ (○), $10 \mu\text{M}$ (△) and $50 \mu\text{M}$ (▽) peptide.

on the elongation arrest mediated by SRP in the synchronized wheat-germ translation system (Fig. 6, tracks 1 and 2); SRP decreased the amount of ^{35}S -preprolactin biosynthesized to 24%. The ovalbumin sequence, on the other hand, reversed SRP-mediated elongation arrest when present at $25 \mu\text{M}$, giving 75% of the translation observed in the absence of SRP.

Electrophoresis of Bolton–Hunter-labelled and photolabelled proteins

Rough microsomal proteins cross-linked by a ^{125}I -labelled photoreactive signal peptide are shown alongside SRP subunits labelled by Bolton–Hunter reagent at a

Fig. 6. Effect of ovalbumin signal peptide on elongation arrest

A fluorograph is shown of ^{35}S -preprolactin translated from bovine pituitary mRNA in a wheat-germ lysate performed in the presence of $25\ \mu\text{M}$ of a control PTH peptide or the signal peptide of ovalbumin (Ov) with or without SRP ($5\ \mu\text{g}/\text{ml}$). Edeine was added 3 min after translation as described for Fig. 5. Also shown is the trichloroacetic acid-precipitated radioactivity as a percentage of that translated without SRP.

ratio of 1 mol of reagent/mol of subunit after separation by SDS/PAGE in Fig. 7. The predominant cross-linked proteins in microsomal membranes are, as reported previously, of 45 kDa (track *a*), and of different molecular mass from the subunits of SRP (track *b*). Weakly labelled cross-linked rough microsomal proteins of 70 kDa, 56 kDa and 14 kDa are also seen (track *a*). As reported previously (Walter *et al.*, 1981), the 72, 68, 54 and 19 kDa subunits of SRP are labelled by the Bolton–Hunter reagent, whereas the lower-molecular-mass subunits are not. Isolated SRP cross-links weakly with the photoreactive signal peptide, and labelled bands appear at both 70 and 56 kDa (Fig. 7, track *c*); the corresponding silver-stained profile of SRP is shown in track *d*. Considering that the covalent addition of the signal peptide to a protein would cause the resulting complex to have a molecular mass increased by 2 kDa, these labelled proteins would be those expected to arise from cross-linking the peptide to the 54 kDa and 68 kDa subunits of SRP.

2D-PAGE of SRP radioiodinated by the Bolton–

Fig. 7. Proteins of rough microsomal membranes and subunits of SRP cross-linked with photoreactive signal peptide

Proteins in salt-stripped pancreatic microsomes cross-linked to ^{125}I -azidophenacyl-signal peptide by u.v. illumination (track *a*) were compared with Bolton–Hunter- ^{125}I -labelled subunits of SRP (track *b*) by autoradiography after separation on a gradient gel. In track (*c*) are shown the subunits of SRP ($2\ \mu\text{g}$ of protein loaded on the gel) cross-linked to the ^{125}I -azidophenacyl-signal peptide, and track (*d*) is the corresponding silver-stained polyacrylamide gel showing protein subunits of SRP.

Hunter reagent is shown in Fig. 8(*a*). A track on the right-hand side of the gel (Fig. 8*a*) shows the labelled SRP subunits run in the second dimension only. The labelled 54 kDa component could be clearly distinguished as a discrete protein with very basic pI, moving to the end of the pH gradient ($\text{pI} > 8.3$) (Fig. 8*a*). In contrast, both the 68 and 72 kDa subunits are relatively acidic (pI 5.1 and 5.7 respectively) and were less easily identified in the Coomassie-Blue-stained protein profile, owing to being partially masked by more abundant proteins of similar molecular mass and similar pI. The 19 kDa subunit has a pI of about 7. Previous 2D-PAGE allowed classification and numbering of the major proteins of rough microsomes (Kaderbhai & Austen, 1984). In terms of the numbering system reported in that earlier paper, the labelled 72 kDa subunit is seen to be located directly under a glycoprotein previously numbered 9, whereas the labelled 68 kDa subunit migrates to the top left corner of a protein labelled 8.

The 2D-PAGE separation of the microsomal membrane proteins photolabelled by the signal-peptide reagent shows that the major cross-linked proteins produced of molecular mass 45 kDa are basic, with pI 7.5–8.0, but less basic than the SRP 45 kDa subunit. The 70 kDa minor protein cross-linked to signal peptide migrates to a pI identical with that of the 68 kDa SRP subunit, whereas the 57 kDa cross-linked protein, in contrast with the highly basic 54 kDa SRP subunit, migrates as an acidic protein (pI 5). From this electro-

Fig. 8. Analyses of Bolton-Hunter-labelled SRP and microsomal cross-linked proteins by 2D-PAGE

Shown are autoradiographs of 2D-PAGE separations of ^{125}I -SRP (2.5 μl) together with pancreatic rough microsomes (100 μg of protein) (a) and proteins of microsomal membranes (100 μg) cross-linked with ^{125}I -photoreactive signal peptide (b). The 54, 68, 72 and 19 kDa SRP subunits are labelled A, B, C and D respectively. The positions of migration of proteins numbered 8, 12, 21 and 22 as in Kaderbhai & Austen (1984) are marked with arrows. Abbreviation: IEF, isoelectric focusing.

phoretic evidence it appears that in microsomal membranes only the 68 kDa subunit of SRP cross-links to the signal peptide.

DISCUSSION

SRP is a naturally occurring component of pancreatic microsomal membranes and reticulocyte lysates, but is reported to be absent from lysates derived from wheat germ (Meyer, 1985). Synthetic signal peptides have previously been found to inhibit processing of newly synthesized secretory protein by added pancreatic microsomal vesicles in a rabbit reticulocyte translation system, probably by competing with precursor proteins for specific components of the secretory apparatus (Austen *et al.*, 1984; Majzoub *et al.*, 1980). In the present studies,

we have found that a tryptic fragment bearing the signal sequence of ovalbumin inhibits processing both in a reticulocyte lysate and in a wheat-germ translation system. There are a number of components of the translocation system with which signal peptides could interact. Inhibition of signal peptidase would decrease the extent of processing of preprolactin seen. However, we found previously that, rather than inhibiting the protease, signal peptides prevent access of pre-proteins to the microsomal lumen, in which the active site of signal peptidase is confined (Austen *et al.*, 1984). Moreover, the consensus signal peptide has been found to have no effect on processing of pre-proteins by microsomal membranes which have been solubilized by detergent, permitting access to the active site of signal peptidase (Robinson, 1987).

Detection of inhibition by signal peptides of processing in the wheat-germ lysate contrasts with the observations by Duong *et al.* (1987), who did not detect inhibition of processing of preprolactin or growth hormone with a synthetic signal peptide resembling the precursor-specific signal region of PTH in a wheat-germ lysate. It is possible, however, that if those workers had examined a wider range of peptide concentrations, then inhibition might have been observed. SRP is present both in the reticulocyte lysate and in the microsomal membranes added, and, as inhibition of processing is observed in both translation systems, it is difficult to deduce with confidence that the signal peptide is interacting with SRP in the reticulocyte lysate or in the added microsomes, or directly with other membrane-associated or soluble components of the translocation systems.

Signal peptides inhibit translation of prolactin and globin mRNA in a rabbit reticulocyte lysate, principally by interfering with polypeptide elongation. Signal peptides inhibit translation of preprolactin in a wheat-germ lysate, but less so than in the mammalian system (Fig. 2). Inhibition was partly reversed by the presence of microsomal membranes. Duong *et al.* (1987) previously interpreted the translation inhibition caused by signal peptides from the precursor region of PTH as being due to SRP-induced elongation arrest imposed by the added peptide. If this interpretation were true, then the increase of translation that we observed on addition of microsomal membranes to the peptide-containing lysate could be due to a reversal of SRP-imposed elongation arrest by docking protein in the microsomal membranes, which has been shown previously to terminate the binding of SRP to arrested polysomes (Gilmore *et al.*, 1982). However, other explanations are possible. The signal peptides may inhibit the action of other functional components of the reticulocyte lysate required for protein chain elongation, causing inhibition of the amount of ^{35}S -globin, a non-secretory protein, and ^{35}S -preprolactin produced. Alternatively, addition of microsomal membranes, which are likely to contain additional receptors for signal peptides (Robinson *et al.*, 1987), may have decreased the inhibitory concentrations of peptide present and consequently increased the amount of protein translated. It should be noted that, in keeping with this interpretation, the concentrations at which translation inhibition is imposed are somewhat higher than those required to block processing (Austen *et al.*, 1984).

At low concentrations (5 μM) of peptide, the elongation arrest imposed by SRP is reversed, whereas at higher concentrations the peptide progressively inhibits translation in addition. Duong *et al.* (1987) came to the conclusion that signal peptides enhance the elongation arrest imposed by SRP, but examined only a narrow range of peptide concentrations. SRP-independent inhibition of protein translation exerted by peptides at higher concentrations would mask any reverse of elongation arrest.

Alkylation of the 54 kDa subunit of SRP has previously been shown to yield a modified ribonucleoprotein particle that fails to recognize signal sequences (Siegel & Walter, 1988). The mechanism of protein elongation arrest may involve binding of the signal sequence to the 54 kDa subunit (Krieg *et al.*, 1986), preventing further movement and elongation of the nascent chain. The signal sequence contained in the nascent chain may be displaced from its binding site on SRP by isolated signal peptides. The

nascent chain would then be free to move on the ribosome, and elongation would continue. However, it has been shown that elongation arrest is not a prerequisite for translocation of pre-proteins across the microsomal membrane and subsequent processing by signal peptidase (Siegel & Walter, 1988). Thus peptide inhibition of translocation may be mediated by interactions with other receptor components of the translocation system *in vitro*.

One candidate for a second receptor for signal sequences could be an additional subunit of SRP. SRP has been shown to retain pre-proteins in a translocation-competent conformation, possibly through a binding site for the signal sequence additional to that present on the 54 kDa subunit (Sanz & Meyer, 1988). It would appear from our present studies that a photoreactive signal peptide irradiated together with isolated SRP cross-links to both the 68 kDa and 54 kDa subunits of SRP. When microsomal membranes are photoreacted together with the photoreactive signal peptide, analysis by 2D-PAGE shows that cross-linking to 45 kDa integral membrane proteins and to a protein with the mobility of the 68 kDa subunit of SRP results, but that no cross-linked protein with the molecular mass and isoelectric properties expected from linking to the 54 kDa subunit was seen. Sequencing of the 54 kDa subunit has recently revealed a potential domain consisting of amphiphilic helices, which could provide a binding domain for signal sequences, as well as a GTP-binding domain (Bernstein *et al.*, 1989). It would appear from our studies that cross-linking of the signal sequence to the 54 kDa subunit is prevented by the presence of microsomes. A possible explanation is that there is very low affinity of the signal sequence for the 54 kDa subunit of SRP in the presence of docking protein contained in microsomal membranes, possibly by modulation through the extent of GTP bound (Gilmore *et al.*, 1982; Bernstein *et al.*, 1989), and so no cross-linking occurs. Alternatively, a covalent link between the 54 kDa subunit and consensus signal peptide is formed, but the resultant complex is so basic that it migrates completely off the gel.

An alternative candidate for a second receptor is the 45 kDa integral membrane protein present in microsomal membranes that is cross-linked relatively strongly by the photoreactive signal peptide (Fig. 7; Robinson *et al.*, 1987). Small pre-proteins such as prepro-mellitin, the frog prepropeptide GLa and the M13 coat protein are known to be translocated by mechanisms that by-pass SRP (respectively: Zimmermann & Mollay, 1986; Schlenstedt & Zimmermann, 1987; Watts *et al.*, 1983). A signal-peptide receptor integrated into the microsomal membrane could provide a recognition point within the cell for smaller pre-proteins so that they could be successfully targeted through the secretory pathway.

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