Interaction of wild-type signal sequences and their charged variants with model and natural membranes

Nalam Madhusudhana RAO and Ramakrishnan NAGARAJ

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

The interaction of synthetic peptides corresponding to wild-type signal sequences, and their mutants having charged amino acids in the hydrophobic region, with model and natural membranes has been studied. At high peptide concentrations, i.e. low lipid/peptide ratios, the signal peptides cause release of carboxyfluorescein (CF) from model membranes with lipid compositions corresponding to those of translocation-competent as well as translocation-incompetent membranes. Interestingly, mutant sequences, which were non-functional *in vivo*, caused considerable release of CF compared with the wild-type sequences. Both wild-type and mutant signal sequences perturb model membranes even at lipid/peptide ratios of 1000:1, as indicated by the

activities of phospholipases A₂, C and D. These studies indicate that such mutant signals are non-functional not because of their inability to interact with membranes, but due to defective targeting to the membrane. The signal peptides inhibit phospholipase C activity in microsomes, uncouple oxidative phosphorylation in mitochondria and increase K⁺ efflux from erythrocytes, and one of the mutant sequences is a potent degranulator of the mast cells. Both wild-type and mutant signal sequences have the ability to perturb vesicles of various lipid compositions. With respect to natural membranes, the peptides do not show any bias towards translocation-competent membranes.

INTRODUCTION

Considerable attention has been focused in recent years on the role of signal sequences in the export of proteins in eukaryotes and prokaryotes [1–4]. A characteristic feature in all signal sequences is the presence of a positively charged N-terminus followed by a segment composed of hydrophobic amino acids [1–4]. An intact hydrophobic stretch is absolutely essential for a functional signal sequence. Reduction of the length of the hydrophobic stretch [5,6], replacement of apolar amino acids with polar analogues [7] and introduction of charged amino acids into the hydrophobic region [8,9] all render a signal sequence non-functional. The hydrophobic nature of signal sequences and the importance of an intact hydrophobic region for a signal sequence to be functional has led to proposals of models for secretion in which translocation across membranes is initiated by partitioning of signal sequences into the lipid bilayer [10–13]. Several protein components of the cells' export machinery have been identified [14–20], and the interaction of signal sequences with these components is likely to be an important step in targeting to translocation-competent membranes. In spite of the absence of a consensus in primary and secondary structures, signal sequences exhibit a remarkable unity of function [21]. Conformational studies indicate that signal sequences can adopt both α -helical and β -structures [22–29]. Many random sequences having the basic architecture of a positively charged N-terminus followed by a hydrophobic region can functionally replace a naturally occurring signal sequence [30]. Although translocation of a polypeptide through the lipid bilayer would be a thermodynamically unfavourable process, protein transport has indeed been demonstrated into proteoliposomes [31–33] and also into vesicles devoid of proteins, except for the signal peptidase, in certain cases [34]. Several laboratories have demonstrated the essential role played by anionic phospholipids in the import of proteins into mitochondria and the export of proteins in bacteria [35–39]. These observations again support the argument

that signal-sequence–lipid interactions are likely to have a role in protein transport. Several mutant non-functional signal sequences have been identified by genetic methods [40], and most of these sequences have a charged residue in the hydrophobic region. It is not clear why these mutant signal sequences are non-functional, or at which step export is affected.

In order to quantify the interfacial behaviour of membrane-active peptides, release of internal markers from vesicles and pressure–area isotherms have been used extensively [29,41–46]. Some of the problems associated with vesicles and monolayers are greater or absence of curvature, loss or lack of asymmetric distribution of lipids, and absence of all of the membrane-associated components that are present in cells and organelles. In spite of such deficiencies, these methods were extremely useful for comparison of the interfacial behaviour of peptides. Interaction of mitochondrial protein presequences with mitochondria was studied by monitoring the ability of these peptides to uncouple mitochondrial oxidative phosphorylation and also to perturb model membranes [39]. It has been suggested that perturbation of the phospholipid bilayer may be part of the mechanism by which mitochondrial presequences initiate import into mitochondria. With several membrane-active peptides, excellent correlations between assays such as internal marker release in model membranes, and enhancement of phospholipase A₂ activity, cytolytic activity and mast cell degranulating activity have been observed [47]. Thus monitoring of one or several membrane-associated functions of the organelles would aid in substantiating the results obtained with simple vesicle content release assays. With signal peptides, such assays will also allow the evaluation of the strength of interactions with target and non-target membranes. To assess the influence of charged amino acids in the hydrophobic stretch in the interaction of the peptide with membranes, two wild-type sequences and corresponding charged analogues were synthesized (Table 1). Genetic studies have indicated that the mutant signal sequence 4 initiates translocation of alkaline phosphatase very inefficiently [48].

Table 1 Primary structures of synthetic signal peptides

Peptides 1 and 2 correspond to signal sequences of chicken lysozyme and *E. coli* alkaline phosphatase. Peptides 3 and 4 correspond to the variants of 1 and 2 respectively, each having a charged amino acid in the hydrophobic region.

Peptide	Sequence
1	Met-Lys-Ser-Leu-Leu ⁵ -Ile-Leu-Val-Leu-Cys ¹⁰ -Phe-Leu-Pro-Leu-Ala ¹⁵ -Ala-Leu-Gly
2	Lys-Gln-Ser-Thr-Ile ⁵ -Ala-Leu-Ala-Leu-Leu ¹⁰ -Pro-Leu-Leu-Phe-Thr ¹⁵ -Pro-Val-Thr-Lys-Ala ²⁰
3	Lys-Leu-Leu-Ile-Ala ⁵ -Leu-Val-Leu-Lys-Phe ¹⁰ -Leu-Pro-Leu-Ala-Ala ¹⁵ -Leu-Gly
4	Lys-Gln-Ser-Thr-Ile ⁵ -Ala-Leu-Glu-Leu-Leu ¹⁰ -Pro-Leu-Leu-Phe-Thr ¹⁵ -Pro-Val-Thr-Lys-Ala ²⁰

Likewise, peptide 3 also would be expected to be non-functional [8]. Interaction of these peptides with model and natural membranes was studied by measuring their ability to affect internal marker release, phospholipases A₂, C and D activities (model membrane assays), inhibition of phospholipase C activity with microsomes, and stimulation of ATPase activity, uncoupler activity (mitochondrial), K⁺ release from erythrocytes and histamine release from mast cells.

MATERIALS AND METHODS

Materials

5,6-Carboxyfluorescein (CF), ATP, ADP, egg phosphatidylcholine, Tris, histamine, mastoparan, melittin, 2,4-dinitrophenol and phospholipase C (from *Bacillus cereus*) (EC 3.1.4.3) were purchased from Sigma. Phospholipase A₂ (hog pancreas) (EC 3.1.1.4) and phospholipase D (cabbage) (EC 3.1.4.4) were purchased from Boehringer. *o*-Phthalaldehyde (Dionex) was from Pierce Chemical Co., and L-phosphatidyl[methyl-¹⁴C]choline 1,2-dipalmitoyl and 1-palmitoyl-2-[1-¹⁴C]palmitoyl-phosphatidylcholine were purchased from Amersham. All other reagents were of analytical grade.

Methods

Small unilamellar vesicles (SUV) were prepared by sonicating the swollen lipid film in 5 mM Hepes buffer at pH 7.4 until clear. The sonicated vesicles were centrifuged (10000 *g*, 10 min) to remove any contamination by large vesicles. Total lipids were extracted from red blood cells, microsomes, mitochondria and *Escherichia coli* by the method of Folch [48a]. Lipid was quantified by phosphate estimation after complete hydrolysis, as described previously [49]. Vesicle disruption was monitored by measuring the release of the entrapped CF as described previously [50], using a Hitachi 650-10S spectrofluorimeter with a stirrer attachment.

K⁺ release from the rat erythrocytes was monitored by the procedure given previously [51] using a K⁺-sensitive electrode (# 93-19) with an Orion EA-940 ion analyser at room temperature. Standard curves using known amounts of K⁺ were constructed each day in the same buffer solutions as were used for the assays, and were utilized to calculate the K⁺ efflux rates.

Mitochondria were isolated from rat liver by the procedures described previously [52]. Oxygen consumption was monitored using a Gilson 5/6 oxygraph fitted with a Clark-type oxygen electrode (Yellow Springs Instruments) at 37 °C. Peptides were added after some State 4 respiration had progressed. The ADP/O ratio was between 2.5 and 2.8 when glutamate and malate were provided as oxidizable substrates. ATPase activity in the rat liver mitochondria was measured by the procedures described previously. [52]. Microsomes from rat liver were isolated as described

previously [14]. The activities of phospholipases A₂, C and D were estimated by the procedures described by Rao [53]. The synthesis of the peptides listed in Table 1 has been described previously [24,26].

Mast cells from the rat peritoneal cavity were isolated by the procedure given previously [54]. Released histamine was estimated by the procedure described by Shore [55].

Protein concentrations were measured by the method of Lowry et al. [56]. Peptide concentrations were estimated by hydrolysing the sample and measuring the amino acid composition using a Pharmacia-LKB Alphaplus amino acid analyser.

CFY rats were used in all our experiments, and were fed *ad libitum*.

RESULTS

Release of CF from vesicles in the presence of peptide

An increase in CF fluorescence occurs on efflux from vesicles, where it is entrapped at a self-quenching concentration, due to dilution. This indicates a change in membrane permeability. The extent and rate of release is indicative of the degree of perturbation of membrane by the peptides. To investigate whether the interaction of the peptide with the membranes is lipid-specific, we have studied the release of CF from vesicles composed of total lipids from microsomes, bacteria, mitochondria and erythrocytes in the presence of signal sequences. The plateau value reached after each addition of the peptide to the vesicle was plotted as a function of the peptide concentration (Figure 1). All the peptides were effective in causing CF release from all four vesicle preparations. The rate of release was similar at all peptide concentrations, and only the extent of release was different. The CF release profiles were essentially linear, indicating the non-cooperative nature of the interaction. Peptide 3 was the most effective in perturbing the lipid vesicles. Such behaviour seems to be in accordance with the fact that a positive charge in a peptide increases the interaction with the membrane, independent of membrane composition. Mutant sequences in all cases were more effective in causing the CF efflux than were wild-type sequences. In spite of wide variations in the lipid composition of these vesicles, no apparent lipid specificity was observed with any peptide. No bias towards model membranes whose lipid composition corresponded to that of the target membranes were observed.

Activity profiles of phospholipase A₂, C and D with peptide-treated egg phosphatidylcholine SUV

In order to assess the interaction of the peptides with the lipid bilayer at lipid/peptide ratios where no CF release was discernible and fusion or aggregation was not detected, the activities of phospholipases with lipid vesicles in the presence of the peptides

Figure 1 Assay of vesicle disruption by peptides, by measuring the release of trapped CF

CF release was measured from SUV prepared with microsomal (a), mitochondrial (b), bacterial (*E. coli*) (c) or erythrocyte (d) total lipids in the presence of each of the four peptides. Plateau values reached after addition of peptide are plotted against the peptide concentration ○, Peptide 1; △, peptide 2; ▲, peptide 3; ●, peptide 4. Lipid concentrations used were 50 μM in case of microsomal lipids and 100 μM in the other three lipid vesicle preparations. 100% release of CF was obtained in the presence of 0.1% Triton X-100. Dimethyl sulphoxide, the solvent for the peptides, did not cause any detectable marker release.

were determined. Monitoring the activities of phospholipases A₂, C and D in SUV with phosphatidylcholine as substrate in the presence of membrane-active peptides is a very sensitive method of directly assessing structural perturbation of the lipid bilayer [53]. We used phosphatidylcholine as substrate because this lipid is predominant in rough endoplasmic reticulum membranes, which the signal sequences encounter *in vivo* in eukaryotes. Also, eukaryotic signal sequences are recognized in prokaryotes and *vice versa* [57,58]. Figures 2(a)–2(f) show the activities of phospholipases A₂, C and D with SUV in the presence of signal peptides 1–4. With both wild-type and mutant signal peptides, the activity of phospholipase A₂ over the control was enhanced, as judged by the increase in hydrolysis (Figures 2a and 2d). Enhanced activity in the presence of peptides 2 and 3 compared with peptides 1 and 4 indicates that the former perturb the lipid bilayer structure more than the latter. Even at a lipid/peptide ratio of 1000:1, the perturbation caused affected the activity of phospholipase A₂. The trend observed with phospholipase A₂ was also seen with phospholipases C and D (Figures 2b, 2c, 2e and 2f). However, with these phospholipases, a decrease in activity is observed with increasing concentrations of peptide. Thus the extent of the decrease in phospholipase activity is a measure of bilayer perturbation. In the case of the alkaline phosphatase signal sequences, the wild-type peptide appears to perturb the lipid bilayer to a greater extent than the mutant peptide. The reverse is true for the lysozyme signal sequences.

Secretory protein signal sequences come into contact with microsomal membranes and the bacterial inner membrane, but not with other cellular membranes. We have studied the membrane interactions of peptides 1–4 by monitoring their effects on membrane-associated functions of mitochondria, mast cells and

erythrocytes. These cells/organelles serve as natural translocation-incompetent membranes in our studies, and have been used extensively in an attempt to understand the mode of action of membrane-active peptides [47,59–61]. Hence we studied the interactions of signal peptides with microsomes, mitochondria, erythrocytes and mast cells.

Interaction of signal sequences with microsomes

To complement the observations made in studies on phospholipases with model membranes, we have chosen to study the interaction of peptides 1–4 with rat liver microsomes by measuring the activity of phospholipase C in peptide-treated microsomes. The advantage of using phospholipases is that the lipid perturbation in the lipid bilayer *per se* can be monitored, unlike several other assays in which the consequences of such a perturbation (e.g. permeability changes) are studied. The modulation of enzyme activity in the presence of the peptides is depicted in Figure 3. It is evident that both wild-type and mutant peptides modulated the enzyme activity. The results indicate that association of signal sequences with the microsomes results in perturbation of the lipid structure, thus affecting the activity of the enzyme.

Effect of signal sequences on mitochondrial functions

In order to study the interactions of signal sequences with mitochondria, we monitored the ability of the peptides to uncouple oxidation of the substrate from phosphorylation and ATPase activity in intact mitochondria. If signal sequences cause proton leakage during oxidative phosphorylation, they would

Figure 2 Activities of phospholipases A₂, C and D with phosphatidylcholine vesicles in the presence of the peptides

Activities of phospholipases A₂ (a, d), C (b, e) and D (c, f) with PC vesicles are shown in the presence of peptides. Activities were determined radiometrically and the data are presented as ratios of activities in the presence and in the absence of the peptide. Data with peptides 1 (○) and 3 (●) are presented in (a)–(c), and data with peptides 2 (○) and 4 (●) are given in (d)–(f). The lipid concentration in all assays was 2 mM.

Figure 3 Activity of phospholipase C with rat liver microsomes in the presence of the peptides

The percentage inhibition of phospholipase activity is shown. The peptide/lipid ratio was 1:50 for peptides 1 and 3 and 1:30 for peptides 2 and 4.

uncouple phosphorylation-controlled oxidation in mitochondria, which would simultaneously enhance the ATPase activity. Figure 4 shows tracings of oxygen consumption using a Clark-type electrode when peptides were added during State 4 respiration. 2,4-Dinitrophenol was included as a positive control. The extent of stimulation of State 4 respiration in the presence of the peptide compared with the control is shown in Figure 5. ATP hydrolysis

Figure 4 Oxygen consumption by rat liver mitochondria in the presence of the peptides

Tracings of oxygen consumption with glutamate/malate as substrate in the presence of each of the four peptides are shown. Addition of ADP and peptide are indicated. DMSO, dimethyl sulphoxide.

in intact mitochondria under conditions of oxidative phosphorylation was monitored in the presence of the peptides, and the fold stimulation in ATPase activity is given in Table 2. It is clear from these data that the signal sequences cause uncoupling and simultaneous enhancement of ATPase activity, similar to a classical uncoupler such as 2,4-dinitrophenol. However 21P, a peptide corresponding to the hydrophobic region of the signal

Figure 5 Uncoupler activity of signal sequence in rat liver mitochondria

Fold stimulation over the State 4 respiration of rat liver mitochondria is plotted against the peptide concentration: 1 (▲), 2 (●), 3 (□), 4 (△) and 21P (○). Peptide 3 could only be tested at one concentration, since it behaved as a strong uncoupler.

Table 2 Activities of rat liver mitochondrial ATPase in the presence of the peptides

The specific activity of the ATPase is given in mmol of P_i produced/h per mg of protein. Final concentrations of the peptides used are given along with the peptides.

Peptide	ATPase activity (mmol/h per mg)	Stimulation (fold)
1 (16 μ M)	2.62	2.2
2 (16 μ M)	1.52	1.3
3 (8 μ M)	3.63	2.9
4 (8 μ M)	2.37	2.0
21P (16 μ M)	2.98	2.5

sequence of the *E. coli* λ -receptor, which was inactive or marginally active in other assays, was effective in uncoupling mitochondrial oxidative phosphorylation. Roise et al. [39] have shown similar uncoupling activity of the presequence (P25 and P33) of subunit IV of yeast cytochrome oxidase. Comparison of these results with the CF release data from vesicles composed of mitochondrial lipid suggests that peptides which were active in the uncoupling assay were also active in CF release experiments. Though none of the peptides used in the present study are amphiphilic, all of them perturbed both mitochondrial natural membranes and artificial membranes composed of mitochondrial lipids. It thus appears that, for vesicle disruption, a peptide need not be amphiphilic as was suggested earlier.

None of the peptides were bactericidal in a disc diffusion assays, and we could not detect any changes in light scattering of the bacteria.

Effect of signal sequences on K^+ efflux from erythrocytes

Efflux of K^+ was monitored in rat erythrocytes suspended in

Figure 6 K^+ release from rat erythrocytes in the presence of the peptides

A K^+ -selective electrode was used to monitor K^+ release from rat erythrocytes suspended in 0.15 M NaCl and 10 mM Tris/HCl (pH 7.4). Total K^+ release (100%) was obtained by solubilizing the erythrocytes by adding 0.04% Triton X-100 at the end of the experiment. The percentage release of K^+ after each addition of peptide is plotted against the concentration of peptides 1 (△), 2 (●), 3 (▲) and 4 (○).

Figure 7 Effects of peptides on mast cell degranulation

Histamine release from rat peritoneal mast cells in the presence of peptide 3 (●) and mastoparan (○) is plotted against the peptide concentration; 100% release was obtained by boiling the mast cells.

saline in the presence of various amounts of peptides. Plotting the percentage release of K^+ against the peptide concentration (Figure 6) shows that peptides 2 and 3 gave about 20% release, whereas the other two peptides were inactive. None of the peptides were haemolytic with rat or guinea pig erythrocytes. Denaturation of the cytoskeleton network either by heating at 49 °C for 10 min or by diamide treatment did not increase the erythrocytes' susceptibility to haemolysis or K^+ release. Assuming a phospholipid content of 2×10^{-10} mg/cell, the peptide/lipid ratios used in the K^+ release experiments were 1:20–1:100. Though these peptides were effective in promoting CF release from SUV composed of total lipids from erythrocytes, they gave no significant perturbation of intact erythrocytes. These data are in agreement with observations that signal sequences in general are not haemolytic.

Effect of signal sequences on degranulation of mast cells

Histamine release from rat peritoneal mast cells was monitored in the presence of the peptides, and with mastoparan as a positive control (Figure 7). Only peptide 3 was effective in degranulating the mast cells and in fact it was more effective than mastoparan, a potent degranulating peptide. Histamine release in the presence of peptides 1, 2 and 4 was not detected even at concentrations of 80 μ M.

DISCUSSION

The clearly discernible common feature in secretory protein signal sequences, prokaryotic as well as eukaryotic, is a positively charged N-terminal region followed by a hydrophobic segment [1–4]. The importance of an intact hydrophobic region for a signal sequence to be functional has been clearly demonstrated [7–9]. Also, at least one membrane barrier, i.e. the endoplasmic reticulum in eukaryotes and inner membrane in bacteria, has to be crossed in order for secretion to occur. When charged or polar amino acids are introduced in the hydrophobic region or when the length of the hydrophobic region is reduced, signal sequences can no longer bring about secretion [5–9]. In this paper we report the data obtained from assays that reflect the interaction of two wild-type sequences and their charged variants with model and natural membranes which included both target and non-target membranes.

At high peptide concentrations, i.e. low lipid/peptide ratios, the signal peptides caused release of CF from vesicles having lipid compositions corresponding to those of translocation-competent as well as translocation-incompetent membranes. Vesicles consisting of total microsomal lipids offered the best system for discriminating the membrane-perturbing activity of peptides. Addition of a positive charge in the hydrophobic region of the peptides enhanced their ability to interact with vesicles of total bacterial lipid, which are predominantly negatively charged lipids, and to a comparatively lesser extent with vesicles composed of erythrocyte lipids. Interestingly, mutant signal sequences with a charged amino acid in the hydrophobic region caused a considerably greater release of CF from vesicles of varied composition than did wild-type signal sequences. Hence it is unlikely that mutant signals with charged amino acids in the hydrophobic region are non-functional because of their inability to interact with membranes. It is likely that precursor proteins with mutant signals are not targeted to membrane sites due to their inability to interact with the components of the transport machinery.

Even at low peptide concentrations, i.e. high lipid/peptide ratios, perturbation of lipid vesicles by signal peptides is discernible. In the presence of signal peptides the susceptibility of SUV to phospholipase A_2 was significantly enhanced. However, these membranes were less susceptible to phospholipases C and D. The rate of lipid hydrolysis by these enzymes in the membrane reflects the accessibility of the hydrolysable bonds. The altered lipid susceptibilities to these phospholipases in the presence of the peptides suggest change in the lipid packing. Similar activity profiles were obtained with potent membrane-interacting peptides such as melittin, alamethicin and gramicidin [53]. These peptides were shown to induce non-bilayer/ H_{II} phases in the membrane with a variety of lipids, and the observed stimulation of phospholipase A_2 activity by these peptides was argued on this basis. Phospholipase A_2 was shown to be sensitive to the bilayer packing stresses during phase changes [62]. Several membrane-active peptides of insect and reptile origin, such as mastoparans, bombolittins etc., also stimulate phospholipase A_2

activity [62]. In addition the PhoE (*E. coli* outer membrane protein) signal sequence was shown to induce H_{II} phases in total bacterial lipids [63]. Preliminary studies on phospholipase C activities with lamellar and inverted hexagonal membranes suggested that the activity of PLC is sensitive to the phase (N. M. Rao, unpublished work). Furthermore, the perturbations caused by these peptides were sensitive to phospholipases at lipid/peptide ratios much higher than those used in the CF release experiments, emphasizing the sensitivity of the membranes to peptide-induced alterations. An ability to induce phase changes, which possibly affect the activities of phospholipases, may be the cause of the solute permeability changes, as observed in the CF release studies. Non-bilayer lipid structures in membranes have been implicated in protein secretion [36,63]. Induction of non-bilayer structures by signal sequences, as suggested in the present study, is in accordance with such a mechanism.

The ability of signal sequences to strongly modulate the activity of phospholipase C towards microsomes suggests that they perturb the lipid structure in the microsomal membrane. The decrease in the activity of PLC in the presence of mutant signal peptides supports our contention that mutant signal sequences lack the ability to target proteins to the membranes, although they still have the ability to interact with the membrane. The lysozyme mutant signal sequence, which has a positively charged amino acid in the hydrophobic region, degranulates mast cells more effectively than does mastoparan. The other signal peptides do not have degranulating ability. Thus wild-type and mutant signal sequences associate with a variety of natural membrane systems and possess *de novo* membrane-modifying properties very similar to those of peptides active with model membranes and peptide toxins [64,65].

Although signal peptides cause efflux of K^+ from erythrocytes, they do not lyse the cells. Even the peptide corresponding to the mutant lysozyme signal sequence, which causes a fairly large efflux of K^+ , is not erythrolytic. However, the peptides have the ability to uncouple oxidative phosphorylation in mitochondria. The signal sequence of the λ -receptor, which does not perturb model membranes, exhibits uncoupling activity. The motif of a positively charged N-terminal region followed by a hydrophobic segment can perturb the mitochondrial membrane in addition to the amphiphilic helical and β -sheet structures found in mitochondrial signal sequences [39]. It is thus unlikely that amphipathic structures in mitochondrial signal sequences have evolved specifically to facilitate interactions with mitochondria.

Protein components clearly have an important role in the intracellular sorting of proteins, particularly in targeting of proteins to translocation-competent membranes. It is unlikely that translocation of proteins across membranes or assembly of integral membrane proteins proceeds by spontaneous insertion of a peptide chain into the lipid bilayer, as envisaged by Engelman and Steitz [12]. Once targeting is achieved, translocation is probably initiated by partitioning of the signal sequences initially into the lipid bilayer. Perturbations caused in the lipid bilayer by the signal peptides could then result in rearrangement of proteins in the membranes to form aqueous conducting channels through which proteins can translocate. The presence of aqueous channels has been demonstrated in membrane vesicles derived from the rough endoplasmic reticulum and from plasma membranes of *E. coli* [66]. Recently, a protein termed 'translocation chain-associating membrane protein' (TRAM) has been characterized from microsomes [67]. Cross-linking studies indicate that short translocating chains of different secretory proteins are associated with TRAM, although TRAM does not simulate the translocation of all secretory proteins to the same extent. However, it is not clear whether TRAM forms a channel. Furthermore, there

is no compelling evidence that these channels are also receptors for signal sequences. Also, stop transfer sequences, which form transmembrane segments of integral membrane proteins, are intimately associated with lipids *in vivo*. The interaction of mitochondrial signal sequences, which form amphipathic helical structures with lipid components of membranes, is an important aspect in models for the import of proteins into mitochondria [68,69]. Hence it is likely that the hydrophobic nature of signal sequences and stop transfer sequences has evolved to allow interactions with the lipid component of membranes. In a recent paper it has been shown that the depletion of signal sequence receptor from microsomes has no effect on elongation arrest release, nascent chain targeting, ribosome binding, protein translocation or membrane protein integration [70]. This suggests that the signal sequence receptor is not essential for protein translocation, and that initiation of translocation can be brought about by insertion of signal sequences into the lipid bilayer of the membranes.

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