REVIEW ARTICLE

The role of topogenic sequences in the movement of proteins through membranes

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INTRODUCTION

Many proteins made in the cytoplasm of eukaryotic cells end up in subcellular compartments or are secreted from the cell. These movements have been described as 'intracellular protein topogenesis' (Blobel, 1980). Intracellular topogenesis is achieved by specific sequences located in the initially biosynthesized form of the protein. These topogenic sequences are known to move newly-synthesized proteins to the lumen of the endoplasmic reticulum, into mitochondria or into chloroplasts. Topogenic sequences also act to move proteins to specified locations within a subcellular compartment, e.g. to move mitochondrial proteins to the mitochondrial outer or inner membrane, the intermembranous space or the matrix, and chloroplast proteins to the outer or inner membranes of the envelope, the intermembranous space, the stroma, or the thylakoid membrane or lumen. In bacteria, proteins made in the cytoplasm transfer to the inner membrane, the periplasmic space or the outer membrane depending on their topogenic sequences.

The purpose of this review is to compare the structure and function of well-characterized topogenic sequences that target newly-synthesized proteins to subcellular compartments in cells and lead to transfer of all or part of the proteins across a membrane. In addition we shall discuss the function of components of cells that have been shown to be involved in the targeting and translocation steps. Identification of topogenic sequences has come from the application of gene fusion technology where the attachment of a topogenic sequence to a normally cytoplasmic protein such as globin, dihydrofolate reductase or β -galactosidase reroutes that protein to the compartment directed by the topogenic sequence.

Secretory signal sequences

The most closely studied sorting sequences are the *N*-terminal transient hydrophobic signal sequences that transport proteins through the membrane of the rough endoplasmic reticulum at the initial stages of the secretory pathway (Blobel & Dobberstein, 1975), act to assemble proteins traversing the membrane by moving hydrophilic domains to the luminal side of the membrane of the endoplasmic reticulum, from where some proteins are moved to the plasma membrane (Rothman & Lodish, 1977) or initiate transport to lysosomes (Erickson & Blobel, 1979).

The signal sequences that export proteins to the periplasm or assemble proteins into the outer membrane in bacteria are structurally similar, and may operate by different but related mechanisms. Many proteins that are assembled into the cytoplasmic side of the endoplasmic

reticulum membrane (e.g. cytochrome b_5) or the inner membrane of bacteria are not made with cleavable signal sequences, and as these proteins are often very hydrophobic they may directly partition into the membrane, and become integrated via hydrophobic insertion sequences (Silhavy *et al.*, 1983).

Studies of fused proteins containing secretory signals

Lingappa *et al.* (1984) showed that a fused protein containing the first 24 residues of the initially translated form of a secreted protein, β -lactamase, directs α -globin across membranes isolated from rough endoplasmic reticulum. Conversely, deletion of the signal peptide of the influenza virus haemagglutinin results in conversion of a glycosylated membrane protein into a cytoplasmic protein (Gething & Sambrook, 1982).

The *N*-terminal sections of several prokaryotic membrane proteins will reroute the cytoplasmic protein β -galactosidase to the outer membrane, but attachment of signals for periplasmic proteins to β -galactosidase results in the fused protein getting stuck in the inner membrane and jamming export of other proteins (Michaelis & Beckwith, 1982). Cleavable *N*-terminal signal sequences are not, then, always sufficient for secretion and β -galactosidase may contain sequences which act to retain it in the membrane. There are similarities in structure and function between bacterial signal sequences and eukaryotic signals that function at the endoplasmic reticulum membrane, and a prokaryotic signal sequence fused to α -globin was shown to be recognized, translocated and processed by eukaryotic endoplasmic reticulum membranes (Lingappa *et al.*, 1984). In general, greater efficiency of secretion of genetically engineered eukaryotic proteins is achieved by attaching a prokaryotic signal sequence to a eukaryotic protein such as growth hormone to obtain high levels of export of the active hormone to the periplasmic space (Becker & Hsiung, 1986).

Position of secretory signals in the polypeptide chain

Most are positioned at the *N*-terminal end of the initially translated form of the protein. Of natural secretory proteins, ovalbumin was the first shown to contain an internal signal sequence which, after a mistaken assignment between residues 229 and 279 (Lingappa *et al.*, 1979), has been located by analysis of a series of deleted forms of ovalbumin produced by directed genetic recombination as lying between residues 22 and 41 (Tabé *et al.*, 1984) in mature ovalbumin. A fused protein containing this section of ovalbumin ligated to α -globin was shown to be moved to the

endoplasmic reticulum lumen in oocytes. A tryptic peptide (residues 21–47) containing the signal region has been isolated from crystalline ovalbumin by a simple h.p.l.c. procedure, and shown to inhibit processing of preprolactin in a translation system *in vitro* supplemented with membranes from the endoplasmic reticulum (Robinson *et al.*, 1986).

Some signal sequences appear to act equally well when relocated to an internal position in the polypeptide chain by genetic manipulation. For example, Perara & Lingappa (1985) made a globin–preprolactin hybrid and showed that the internal preprolactin signal is recognized by endoplasmic reticulum membranes *in vitro*. Globin with the signal at its C-terminus and mature prolactin were released by cleavage.

Signals that assemble proteins into membranes

Three types of membrane-spanning proteins have been identified. Those spanning the membrane once, with their C-termini exposed to the cytoplasm (type I), are usually synthesized with cleaved signal sequences and are held in the membrane by a second hydrophobic 'stop-transfer' domain. Examples include the vesicular stomatitis virus G protein (Lingappa *et al.*, 1978). Type II membrane proteins have their N-termini exposed to the cytoplasm, and are synthesized without cleaved signals. It has been shown for the human transferrin receptor (Zerial *et al.*, 1986) that the membrane-spanning region functions as a signal sequence, because fused proteins containing the membrane-spanning domain ligated to dihydrofolate reductase or globin are assembled into microsomal membranes *in vitro* in the presence of the Signal Recognition Particle (SRP), an 11 S ribonucleoprotein isolated from rough microsomal membranes by Walter & Blobel (1981), the properties of which are described in more detail below. Rat liver asialoglycoprotein receptor (Holland & Drickamer, 1986) and the invariant chain of class II histocompatibility antigen (Bos *et al.*, 1984) also lack cleavable signal sequences and have type II orientation. The third type of membrane protein is polytopic, crossing the membrane several times, and opsin, for example, has been shown to possess more than one signal sequence (Friedlander & Blobel, 1985). In this case, as in others, the definition of a signal sequence is implied from an observed dependence on SRP (see below).

Secretory proteins can be converted to membrane proteins by appending membrane-spanning domains (Sharma *et al.*, 1986), whilst membrane proteins may be converted to secretory proteins by removal of the membrane-spanning domain, providing the topogenic tertiary structure required for moving the protein to the cell surface is present in the product (Murre *et al.*, 1984; Gething *et al.*, 1986). It appears that it is possible to convert signal sequences to membrane-spanning domains by repositioning. For example, in a fused protein consisting of two bacterial pre-secretory proteins, the second signal acts as a membrane-spanning domain if the distances between the two signals is 27 residues (Coleman *et al.*, 1985), whereas the second acts as a cleavable signal for export if the distance is only 13 residues. In contrast, Yost *et al.* (1983) placed the membrane-spanning domain of the surface immunoglobulin heavy chain 40 residues from the N-terminus of a fused protein and found that it did not provide the signal function. Conversely, the membrane-spanning

domain of the human invariant chain of class II histocompatibility antigens can be converted to a cleavable signal sequence by deletion of hydrophilic residues from its cytoplasmic domain (Lipp & Dobberstein, 1986), the exoplasmic domain being secreted.

STUDIES OF TRANSLOCATION MECHANISMS

The ribosome–membrane junction

In the 1960s it became clear that ribosomes bound to membranes in the rough endoplasmic reticulum of eukaryotic cells made secretory proteins whilst free ribosomes made cytoplasmic proteins (Redman & Sabatini, 1966), indicating that the ribosome–membrane junction observed under the electron microscope was involved in protein secretion. Electron microscopy of isolated rough microsomes shows that the ribosomes are attached only to the outside (cytoplasmic) side of the vesicles. Rough or ribosome-depleted microsomes mimic *in vitro* many of the activities, including translocation, cleavage and glycosylation of nascent secretory protein, associated with the rough endoplasmic reticulum in intact cells.

Puromycin, an inhibitor of protein elongation which acts by premature termination of nascent chains, together with high salt causes release of ribosomes from pancreatic rough microsomes, suggesting that the nascent chain at least in part constitutes the ribosome–membrane junction. As the released nascent chains were found to be internalized into microsomal vesicles *in vitro* it was suggested that during biosynthesis they are vectorially extruded into the lumen of the endoplasmic reticulum across its membrane (Redman & Sabatini, 1966). Blobel & Sabatini (1971) proposed that the N-terminal region of the nascent chain mediates binding of the ribosome to the membrane.

Translation from secretory mRNA *in vitro*

The development of protein translation systems *in vitro* programmed with mRNA for secretory proteins, [³⁵S]methionine and microsomal membranes to study the translocation and processing of the labelled translated secretory proteins have allowed identification of mechanisms and components involved in the targeting, translocation and processing of proteins by these membranes. The vast majority of mRNAs (nearly 300 up to mid-1984; Watson, 1984) for secretory proteins and many integral membrane proteins from a variety of organisms, including bacteria, encode proteins which contain transient signal sequences which are removed at the early stages of biosynthesis.

The rather precise time window early in translation during which addition of microsomes to a synchronized translation of mRNA encoding several different types of secretory or membrane-assembled protein (e.g. vesicular stomatitis G protein, Sindbis PE2 protein, pancreatic secretory proteins, prolactin) results in translocation and cleavage of the product, led to the suggestion that the signal sequence emerging from the exit site on the large ribosomal subunit could only make functional interactions with microsomal membranes if the nascent chain was no longer than about 80 residues (Rothman & Lodish, 1977; Garoff *et al.*, 1978; Scheele *et al.*, 1980; Lingappa *et al.*, 1979).

The signal hypothesis

Blobel & Dobberstein (1975) proposed that the signal peptide as it emerged from the peptidyl site in the large ribosomal subunit of a free ribosome triggered binding of the ribosome to the endoplasmic reticulum membrane and assembly of protein subunits in the membrane to form a pore through which the growing chain passed. After completion of transfer, the ribosome would detach and the protein pore disassemble. The hypothesis has since been modified to incorporate a role for SRP (Walter & Blobel, 1980), and to encompass signal sequences that are not at the *N*-terminus and are not cleaved. It was also suggested that type I membrane proteins are inserted into the membrane by their signal sequences, and translocated vectorially until the hydrophobic 'stop-transfer' sequence is reached, at which point translocation through the membrane ceases, the ribosomes leave the membrane and biosynthesis of the C-terminal cytoplasmic section is completed (Blobel, 1980). The hypothesis does not take into account the hydrophobic nature of the signal sequence, which appears to have an appropriate structure to interact directly with membrane phospholipids, nor the possibility of substantial folding of the polypeptide chain prior to translocation, which would make passage through a protein channel difficult to conceive.

Post-translational movement across the endoplasmic reticulum membrane

It now appears that translocation and processing at the endoplasmic reticulum is less tightly coupled to translation than was first believed. Prepromellitin, a small (70 residue) protein, is imported into microsomes and processed after translation in a rabbit reticulocyte lysate (independent of SRP; see below) (Zimmerman & Molloy, 1986). In rabbit reticulocytes, 25% of preplacental lactogen, the translation of which had been terminated by cycloheximide, was post-translationally processed to its mature form by microsomes, but only those chains that were still attached to ribosomes, possibly via SRP, were processed (Caulfield *et al.*, 1986). Pre- α -mating factor is processed post-translationally in a yeast cell-free translation system by microsomal membranes also derived from yeast (Hansen *et al.*, 1986; Waters & Blobel, 1986; Rothblatt & Meyer, 1986).

By arresting translation of mRNA for IgG light chains and preprolactin by SRP late in the course of elongation, it has been shown that addition of microsomes to nascent chains which were up to 75% of their final length (i.e. 19 kDa) still led to processing (Ainger & Meyer, 1986). Perara *et al.* (1986) eliminated termination codons from cDNA constructs derived by ligating the DNA of the signal sequence of β -lactamase to the DNA of α -globin, and demonstrated post-translational uptake of the resulting hybrid proteins into canine pancreatic microsomes. Only those nascent chains that were still attached to ribosomes were processed post-translationally. Finally, a form of the human glucose transporter protein missing its C-terminal domain was efficiently assembled into canine pancreatic microsomes post-translationally, but the assembly depends upon SRP (Mueckler & Lodish, 1986). It is likely that the signal peptide, at least in longer proteins, must make critical interactions with SRP while emerging from the ribosome for the eventual translocation to be successful.

Energy for translocation at the endoplasmic reticulum

Demonstration of post-translational translocation suggests that models of protein translocation across the endoplasmic reticulum membrane that rely on an input of energy from protein translation (von Heijne & Blomberg, 1979) may not be universally applicable. Because hydrolysis of nucleoside triphosphates also provides energy for polypeptide initiation and elongation, it had not previously been possible to demonstrate dependence of translocation on nucleoside triphosphates during translation. By depleting translation systems of their GTP and ATP after translation, several workers have shown that subsequent post-translational processing is dependent upon readdition of ATP and GTP, or other high-energy phosphates (Hansen *et al.*, 1986; Waters & Blobel, 1986; Rothblatt & Meyer, 1986; Ainger & Meyer, 1986; Perara *et al.*, 1986).

GTP had previously been shown to alter markedly the membrane barrier of stripped rough microsomes from rat liver, leading to a reduction in the latency of mannose-6-phosphatase, and stimulation of the dolichol pathway for protein glycosylation (Godelaine *et al.*, 1983). As GTP acted in this way only when about 60% of ribosomal RNA had been stripped away it was postulated that by interacting with some functional components of the membranes, ribosomes hinder the GTP-dependent increase in membrane permeability. Recently, Connolly & Gilmore (1986) found that an SRP-arrested fragment of preprolactin and its associated ribosome formed a salt-resistant attachment to pancreatic microsomes only in the presence of GTP or its non-hydrolysable analogues, but not with ATP alone. The arrested fragment was highly resistant to the action of added proteases in this complex, indicating close juxtaposition of the fragment to the membrane and the ribosome surface. Subsequent translocation and processing was not however dependent upon GTP or ATP. Although non-hydrolysable GTP analogues were found to be as effective as GTP in stimulating the attachment of the complex to the membrane, further rounds of ribosome detachment and rebinding to membranes may require GTP hydrolysis. A 22 kDa GTP-binding membrane protein has been identified as a possible candidate for a protein involved in nascent chain-ribosome binding in rough microsomes from liver and pancreas (Robinson & Austen, 1987). This protein is labelled by [³²P]ADP-ribosylation in the presence of [³²P]NAD and GTP and catalytic amounts of cholera toxin.

Co- and post-translational movement across the bacterial cytoplasmic membrane

A number of periplasmic (e.g. MalE) and outer membrane (e.g. LamB) proteins are shown to be synthesized preferentially on membrane-bound ribosomes (Randall & Hardy, 1977; Baty *et al.*, 1981). Davis and his colleagues showed that nascent chains are labelled by probes designed to be membrane-impermeant (Smith *et al.*, 1979), but as prolonged incubation revealed that intracellular components were also labelled it appeared that in fact the probes were able to penetrate the membrane. In the cell-free processing system devised by Muller & Blobel (1984) the precursors of alkaline phosphatase and LamB are processed by inverted plasma membrane vesicles post-translationally providing the ATP pool is replenished.

By examining the size distribution of proteolytic cleavage products produced from radiolabelled nascent chains of bacterial secreted or membrane proteins, Josefson & Randall (1981) showed that translocation and signal cleavage is a relatively late event, occurring in some instances when the chains were 80% of completed size, and in some instances after the completion of translation. Randall & Hardy (1986) have shown that a correlation exists between susceptibility to exogenous proteolysis and protein export, indicating that changes in conformation of the precursors that would change their susceptibility may prevent export.

The post-translational export of some proteins is inhibited by chemicals known to dissipate the electrochemical gradient across the inner membrane (Date *et al.*, 1980; Zimmerman *et al.*, 1982). Asymmetric charge rather than proton gradient is required (Pages & Lazdunski, 1982).

The membrane trigger hypothesis

The M13 coat protein is synthesized as a 73-amino-acid precursor, procoat, with a 23 residue signal sequence and is assembled after translation under the influence of the electrochemical gradient across the cytoplasmic membrane and then cleaved in the periplasm (Wickner, 1979). Spontaneous integration into liposomes either with or without the protease that cleaves the signal (signal peptidase) has been observed (Ohno-Iwashita & Wickner, 1983). To explain the assembly of this protein, Wickner proposed that the role of the signal sequence is to promote folding of the completed precursor into a soluble export-competent form. Contact with the hydrophobic environment of the membrane is then envisaged to promote a conformational change which allows spontaneous insertion, while signal peptide cleavage would render the process irreversible (Wickner, 1979).

STRUCTURE OF SIGNAL SEQUENCES

Analyses of a number of signal sequences from prokaryotes and eukaryotes have revealed that, although the amino acid sequences vary considerably, there are certain shared structural features (Austen, 1979; Austen & Ridd, 1981; von Heijne, 1985). Signal sequences are quite variable in length, ranging from 16 to more than 50 residues in length, but the average length is about 20 residues. The principal feature is a central region of between 8 and 18 uncharged consecutive residues (h-region), readily recognized by performing the hydrophobicity analysis described by Kyte & Doolittle (1982). Rapoport (1985) has suggested that membrane-spanning regions contain longer stretches of uncharged residues (at least 18) than signal sequences, although evidence summarized above suggests that they are interchangeable in some situations.

The hydrophobic region is bounded on the *N*-terminal side by a more polar region that generally contains basic residues, and on the *C*-terminal side a more polar region of several residues exists between the hydrophobic core and the site at which signal peptidase cleaves. A model has been suggested whereby the h-region inserts into the membrane by adopting a transmembrane orientation with the *N*-terminal basic residues remaining on the cytoplasmic side of the membrane, translocating the attached chain as a loop (Austen, 1979), and exposing

Table 1. Structures of signal and transit peptides

I, II, and III denote regions of homology (Karlin-Neumann & Tobin, 1986); II contains a potential cleavage site between Gln and Gly. Underlined residues are hydrophobic; † denotes basic residues; * denotes hydroxyl residues.

Signal of β -lactoglobulin	
† Met-Lys-Cys-Leu-Leu-Ala-Leu-Gly-Leu-Ala-Leu-Ala-Cys-Gly-Val-Gln-Ala	
Consensus signal peptide (substituted at the Cys residue with a <i>p</i> -azidophenacyl group to render peptide photoreactive)	†
† Lys-Lys-Ser-Ala-Leu-Leu-Ala-Leu-Met-Tyr-Val-Cys-Pro-Gly-Lys-Ala-Asp-Lys-Glu	
Ovalbumin signal sequence	
† Val-His-His-Ala-Asn-Glu-Asn-Ile-Phe-Tyr-Cys-Pro-Ile-Ala-Ile-Met-Ser-Ala-Leu-Ala-Met-Val-Tyr-Leu-Gly-Ala-Lys	†
Mitochondrial transit peptide (subunit IV of yeast cytochrome oxidase)	†
† Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu	†
Chloroplast transit peptide (small subunit of wheat ribulose 1,5-bisphosphate carboxylase)	†
Met-Ala-Pro-Ala-Val-Met-Ala-Ser-Ala-Thr-Thr-Val-Ala-Pro-Phe-Gln-Gly-Leu-Lys-Ser-Thr-Ala-Gly-Leu-Pro-Ile-	†
† * Ser-Cys-Arg-Ser-Gly-Ser-Thr-Gly-Leu-Ser-Ser-Ser-Val-Ser-Asn-Gly-Gly-Ile-Arg-Cys-Met	†
	I
	II
	III

the cleavage site on the luminal side of the membrane. Adoption of a loop structure would explain why type I and type II membrane proteins with cleaved and uncleaved signals respectively end up in opposite orientation in the membrane.

Predictions of secondary structure and minimum energy calculation have shown high potential for adopting α -helix within the hydrophobic core, with a β -turn close to the cleavage site (Austen, 1979; Garnier *et al.*, 1980; Pincus & Klausner, 1982). The importance of common structural features rather than precise highly conserved sequences is shown by the design and synthesis of a signal sequence that was a consensus of known signal sequences rather than a naturally occurring one (Table 1). This peptide has been shown to compete with a number of different types of signals on newly-biosynthesized secretory proteins *in vitro*, preventing their uptake and processing into microsomal vesicles (Austen *et al.*, 1984).

Kaiser *et al.* (1987) found that one-fifth of random sequences used to replace the normal signal sequence of invertase functioned as export signals, but few of them were cleaved by signal peptidase. Although there was a slight tendency for functional sequences to be hydrophobic, fewer consecutive hydrophobic residues were required than the number found in natural signals. The sequence requirements for cleavage may be more restrictive than for translocation.

Mutations in the signal sequence

Export-deficient mutations resulting from changes in the signal sequence have been identified in bacteria. Most result from decrease in the length or hydrophobic nature of the h-region. Bedouelle *et al.* (1980) isolated five different point mutations in the h-region of the maltose binding protein, four of which were a change to a charged residue, while the fifth was a change to a helix-breaking proline. By analysing the structures of pseudo-revertants of the deletion mutant LamB, missing residues -13 to -16, Emr & Silhavy (1983) suggested that α -helix formation in the h-region was also important. A minimum length of seven hydrophobic residues in the h-region of the maltose binding protein seems necessary for export (Bankaitis *et al.*, 1984). Site-specific mutagenesis in the h-region to produce nine consecutive leucine residues yields a form of alkaline phosphatase that is exported more efficiently than is the wild type (Kendall *et al.*, 1986).

Cleavage of signal peptides

The actual cleavage site shows primary sequence specificity of a '-1, -3' rule (Austen & Ridd, 1981; von Heijne, 1984) in that small neutral residues (alanine, serine, glycine, cysteine, threonine or occasionally glutamine) occur at position -1 (the residue providing the carboxyl of the cleaved peptide bond), and uncharged amino acids occur at position -3. Alterations near or at the cleavage region have ascertained the need for small, uncharged residues at -1 (Schauer *et al.*, 1985) in the precursor of yeast invertase, and for a β -turn assisting proline at -4 in pre- β -lactamase (Koshland *et al.*, 1982).

Cleavage of the signal peptide occurs on the luminal side of the endoplasmic reticulum (Jackson & Blobel, 1977). The early studies of Blobel & Dobberstein (1975) indicated that cleavage was a co-translational event,

although this may require reinvestigation with some pre-proteins in view of recent findings that translocation may be post-translational. Assays that monitor cleavage of 35 S-labelled pre-proteins are generally performed with signal peptidase solubilized from microsomal membranes with non-ionized detergents, and because the purified enzyme requires phospholipids for stability (Jackson & White, 1981) it is thought that the peptidase in the endoplasmic reticulum is closely associated with the membrane. Removal of the cytoplasmic hydrophilic domain of the invariant chain of class II histocompatibility antigen by genetic recombination has been shown to expose the membrane-spanning domain to signal peptidase, and it was proposed that basic residues on the N-terminal side of signals serve to position the signal accurately across the membrane, aligning the potential cleavage site close to the peptidase active site (Lipp & Dobberstein, 1986). However, a 16-residue truncated form of M13 procoat without the basic N-terminal region and most of the hydrophobic region is correctly cleaved *in vitro* by bacterial signal peptidase (Dierstein & Wickner, 1986).

As pre-proteins have quite different properties to their mature forms (Ridd *et al.*, 1982), cleavage of the signal allows proteins to adopt their active configuration, and may be necessary to make movement across the membrane irreversible. An exception is ovalbumin, which contains a signal that is not cleaved (Palmiter *et al.*, 1978), and the inference that cleavage is not essential for translocation has been shown in eukaryotes by blockage of cleavage, but not secretion, by incorporation of the amino acid analogue hydroxynorvaline into nascent preprolactin at the cleavage site (Hortin & Boime, 1981) and in prokaryotes by isolation of mutants that were capable of exporting uncleaved forms of β -lactamase to the periplasmic space (Koshland *et al.*, 1982).

COMPONENTS INVOLVED IN TARGETTING, TRANSLOCATION AND PROCESSING

Signal recognition particle

Salt-extracts of pancreatic microsomal membranes have been shown to contain an 11 S ribonucleoprotein particle which restores the ability of salt-extracted microsomes to translocate and process secretory and membrane-associated proteins in translation systems *in vitro* (Walter & Blobel, 1980). The ribonucleoprotein was purified by chromatography on aminopentyl-agarose, and was found to consist of six protein subunits (72, 68, 54, 19, 14 and 9 kDa) and a 7 S RNA molecule (Walter & Blobel, 1982). Dependence of translocation on SRP has been described for a variety of mammalian secretory and membrane proteins, including those containing signal peptides which are not cleaved, and a few plant proteins.

In a wheat-germ translation system, SRP was shown to arrest the elongation of secretory or membrane nascent proteins (Walter & Blobel, 1981), and it was proposed that this delay allowed functional binding of the polysomal complex to the membrane before the nascent chain reached appreciable length. Although Walter & Blobel (1981) originally showed that arrest of preprolactin occurred as the signal left the ribosome, i.e. when the chain is about 80 residues in length, recent

Fig. 1. Detergent extraction of microsomal proteins photolabelled with an azidophenacyl-signal peptide

EDTA/salt-stripped pancreatic rough microsomes (10 mg of protein/ml) were u.v.-irradiated with ^{125}I -azidophenacyl signal peptide as described by Robinson *et al.* (1987). Aliquots containing 0.2 mg of protein were diluted with equal volumes of 50 mM-KCl/5 mM-MgCl₂/50 mM-Hepes/KOH pH 7.4, containing Nonidet P40 to give the final concentrations shown, vortex-mixed and centrifuged at 440000 *g* for 15 min. Proteins in the supernatants (tracks 2–5) and the pellets (tracks 6–9) were separated by linear gradients of 7–14% polyacrylamide in SDS. The major protein labelled was of 45 kDa, and was extracted by between 0.5% and 1.0% (v/v) Nonidet P40, which is between 4.7 and 9.4 mol of effective detergent/mol of phospholipid, being not completely extracted until the bilayer is solubilized. Ribosomal proteins remain in the pellets (tracks 8 and 9).

studies have shown that chains can be arrested in translation when they are much longer (see above). SRP particles reconstituted without two of its protein subunits, the 9 and 14 kDa subunits, no longer functioned in elongation arrest (Siegel & Walter, 1985), while removal of the *Alu* sequences in the 7 S RNA component reduced elongation arrest considerably; that the modified SRP particles were still able to reconstitute processing to salt-stripped microsomes (Siegel & Walter, 1986) showed that elongation arrest is not a critical part of the targetting of polysomes to the endoplasmic reticulum membrane. Meyer (1985) showed that arrest of elongation occurred only in a wheat germ translation system, and not in a reticulocyte lysate or translation system derived from HeLa cells, so the elongation arrest may simply result from a mis-match between plant ribosomes and mammalian SRP.

Docking protein

Pancreatic or liver rough microsomal membranes contain an integral 72 kDa protein (docking protein) required for their translocation activity. Trypsinization or treatment of rough microsomes with elastase followed by removal of a 60 kDa fragment cleaved from docking protein by salt washing leads to their inactivation in processing systems *in vitro* (Meyer & Dobberstein, 1980). The purified 60 kDa fragment was found to reconstitute translocation to protease-digested microsomes. As this fragment reverses the elongation arrest imposed by SRP,

the 72 kDa protein is thought to be the SRP receptor. The affinity of docking protein for SRP has been demonstrated by its isolation by affinity chromatography on immobilized SRP (Gilmore *et al.*, 1982).

Docking protein is 638 amino acids long with a hydrophobic *N*-terminus divided into two domains, one of which is believed to span the membrane, while the rest of the protein consists of eight hydrophilic regions with an overall basic charge (Lauffer *et al.*, 1985) which may allow its interaction with the 7 S RNA of SRP.

Signal peptide receptor

Synthetic signal peptides block entry of nascent secretory proteins into microsomal membrane vesicles *in vitro* (Majzoub *et al.*, 1980; Austen *et al.*, 1984), and the existence of receptors for signal peptides with K_D of 10^{-7}M has been shown by direct binding studies (Austen & Ridd, 1983). Interestingly, when the nature of this receptor was investigated by u.v.-irradiating rough microsomes in the presence of a highly radioactive signal peptide chemically substituted with a photoreactive azidophenacyl group (Table 1), a 45 kDa labelled protein containing covalently linked signal peptide was identified (Fig. 1). This microsomal protein is different in molecular mass from any of the subunits of SRP (Robinson *et al.*, 1985, 1987). Moreover, the protein is present in salt-washed or alkali-washed rough microsomes from both pancreas and liver, and its release from membranes was achieved only by high concentrations of

Fig. 2. Model of signal-sequence-assisted translocation at the endoplasmic reticulum (a) and model of protein import into mitochondria (b)

(a) After the signal is targetted to the membrane by SRP and docking protein (DP), the binding of the signal peptide to a 43 kDa protein induces GTP binding to a neighbouring membrane protein, which in turn acts to destabilize phospholipids, allowing insertion, translocation and cleavage of the protein by signal peptidase (SP). (b) Outer membrane proteins require no receptor, energy or cleavage for assembly. Apocytochrome *c* is imported by its own receptor, without cleavage or energy input. Precursors of matrix, inner membrane, and intermembranous space (IMS) proteins bind a cytoplasmic factor (TRP), which prevents folding prior to binding to a receptor. Under the influence of the electrochemical gradient, transit sequences move to the matrix, where those of both matrix and IMS proteins are cleaved. IMS proteins are then rerouted to the IMS where a second cleavage occurs.

non-ionized detergent, showing that it is an integral membrane protein (Fig. 1).

Binding to the photoactivatable signal is saturable and specific for signal peptides, and is not displaced by control hydrophobic peptides (Robinson *et al.*, 1987). The microsomal binding protein possesses a cytoplasmic 15 kDa domain which may be proteolytically removed, and proteolysis under the same relatively vigorous conditions (after first removing the 60 kDa fragment of docking protein) has been shown to yield microsomes inactive in their ability to process secretory proteins *in vitro* even after reconstitution of the docking protein. The manner in which an integral membrane receptor could bring about the translocation of the *N*-terminal region of a newly-synthesized protein at the endoplasmic reticulum is depicted in Fig. 2(a).

sec mutants in bacteria

A number of conditionally lethal mutants have been described with defects in bacterial protein export (for reviews see Oliver, 1985; Benson *et al.*, 1985). In one of these strains, which lack the *secA* protein product, there is complete inhibition of export protein synthesis at non-permissive temperatures, although this does not occur with strains that produce export proteins with defects in their signal sequences (Kumamoto *et al.*, 1984). Thus interaction of the *secA* gene product with a signal sequence may be essential for the synthesis of export proteins, and the *secA* gene product, which is a 92 kDa cytoplasmic peripheral membrane protein, has been likened to SRP and may act to couple synthesis to secretion (Kumamoto *et al.*, 1984).

Three second site suppressor mutations, *secY*, *prlB* and *prlC* have been shown to confer wild-type phenotypes to a mutant strain carrying a three amino acid deletion in the hydrophobic region of the LamB signal sequence. In contrast with the *secA* gene, the protein encoded by the *secY* gene can act post-translationally to promote protein export (Bacallao *et al.*, 1986). The *secY* gene is the last gene (72 min) in the *spc* operon (Cerretti *et al.*, 1983) which codes for ten ribosomal proteins. The encoded protein is very hydrophobic, resides in the inner membrane, and is 49 kDa in molecular mass, i.e. an interesting closeness in molecular mass and membrane association to the 45 kDa signal binding protein in microsomal membranes.

***sec* mutants in yeast**

Some temperature-sensitive mutants have been isolated which fail to produce active secretory enzymes at the restrictive temperature (37 °C). In *sec 53* and *sec 59* the rates of protein synthesis are normal but glycosylation is incomplete due to the secretory proteins getting stuck in the endoplasmic reticulum membrane. Although treatment of yeasts with tunicamycin also blocks secretion, the effect is not thermoreversible. The *sec 53* mutant has been cloned and shown to code for an abundant soluble cytoplasmic 28 kDa protein of as yet ill-defined function (Ferro-Novick, 1985).

Ribophorins

The proposal that a pair of integral membrane proteins called ribophorins, which are found only in rough microsomes, act as ribosomal binding proteins (Kreibich *et al.*, 1978) has been questioned by Hortsch *et al.* (1986), who found that proteolysis of microsomes sufficient to destroy ribosome binding *in vitro* leaves ribophorins intact; thus the nature of the electrostatic link between membranes and ribosomes remains unknown.

Signal peptidase

The purification of the enzyme from pancreas has been reported (Evans *et al.*, 1986) and the enzyme appears to be basic in character and to be composed of a complex of six non-identical subunits of molecular masses 25, 23, 22, 21, 18 and 12 kDa, two of which are glycoproteins.

Signal peptidase from *E. coli* has been purified as a single polypeptide of molecular mass 37 kDa (Wolfe *et al.*, 1982). The purified enzyme in detergent correctly cleaves the precursors of a number of preproteins, including the eukaryotic IgG κ chain precursor from mouse (Watts *et al.*, 1983). Like most inner membrane proteins, signal peptidase is made without a signal sequence, and is assembled into the membrane with a large C-terminal domain periplasmic, and a small N-terminal cytoplasmic domain (Ohno-Iwashito *et al.*, 1984).

Little is known of the catalytic mechanism of either the eukaryotic or prokaryotic enzyme.

PHYSICAL PROPERTIES OF SIGNAL PEPTIDES

Syntheses of a number of signal peptides, smaller fragments containing parts of their sequences, or elongated forms containing parts of the mature protein

in addition have been reported (Majzoub *et al.*, 1980; Austen & Ridd, 1981; Austen *et al.*, 1984; Briggs & Gierasch, 1984; Najaraj, 1984). The pressure-area isotherms of the signal sequence of pretrypsinogen 2, a consensus signal sequence and the isolated signal from ovalbumin (Table 1) at an air-water interface indicate that the surface films produced are more stable than those of other proteins, including membrane proteins and mellitin. Moreover, lateral stabilities are increased in the presence of phospholipids. The signal peptides were found not to insert spontaneously from the aqueous phase into a phospholipid monolayer, which may explain why a signal binding protein integrated into the membrane may be required to put the signals into a transmembrane orientation. The reported lower stability of secreted proteins in an environment akin to the interface occurring at a membrane may explain their extrusion from membranes into the lumen of the endoplasmic reticulum, either after cleavage of the signal peptide, or after a conformational change. In keeping with earlier empirical calculations which suggested that secondary structure of signals was likely to be important for their ability to translocate proteins, the observed molecular areas of signal peptides at an air-water interface indicate considerable secondary structure (Fidelio *et al.*, 1986).

Circular dichroism of synthetic signal peptides shows that most are random coil in water and adopt a considerable degree of α -helical structure in solvents that are more apolar (Austen & Ridd, 1981; Briggs & Gierasch, 1984) and it has been suggested that they undergo a conformational change upon entering the membrane. In contrast, a 29-residue peptide consisting of the pre-pro region of parathyroid hormone adopts predominantly β -structure (Rosenblatt *et al.*, 1980). Fourier transform i.r. analysis shows that signal peptides in dispersions with phospholipids adopt conformations that are mainly β -sheet (G. D. Fidelio, P. J. Harris, B. M. Austen, D. Chapman & J. A. Lucy, unpublished work). The concentrations of peptide upon which measurements are made are extremely high, but β -sheet stabilization may be important in polytopic membrane proteins where multiple signal sequences may be at high local concentrations.

TRANSIT PEPTIDES

Transport into mitochondria

Pulse-labelling of yeast spheroplasts and subsequent immunoprecipitation has shown that many mitochondrial proteins are made as cytoplasmic precursors between 0.5 and 10 kDa larger with the additional transit sequences N-terminal (Maccacchini *et al.*, 1979). Although these transit sequences are generally quite long (20–60 residues) (Hay *et al.*, 1984), a small portion (12 residues) of the N-terminus of the initially translated form of cytochrome *c* oxidase, a matrix protein, will reroute mouse dihydrofolate reductase into the mitochondrial matrix (Hurt *et al.*, 1985a). Although transit sequences are generally N-terminal, the extreme N-terminal section is not always the critical region. Horwich *et al.* (1986) have shown that the middle section of the ornithine transcarbamoylase transit sequence is essential for import, whereas the N- and C-terminal sections may be deleted without effect.

Other portions of these transit sequences may sort the imported proteins into different locations within mitochondria. For example, the 61-residue transit sequence of yeast cytochrome *c*₁ is in two domains and is cleaved in mitochondria in two steps. The *N*-terminal 32 residues has been shown to import dihydrofolate reductase to the matrix, whereas the *N*-terminal 64 residues of premature cytochrome *c*₁ locates dihydrofolate reductase to the intermembranous space, and thus it was proposed that the *C*-terminal half of the transit peptide acts as a second topogenic sequence to transfer the protein from the matrix back to the intermembranous space (Adolphus *et al.*, 1986). The *N*-terminal 12 residues of the 70 kDa outer membrane protein when fused to cytochrome *c* oxidase lacking its own transit sequence causes the fused protein to be directed to the matrix side of the inner membrane. The adjacent domain in the outer membrane protein, which consists of 23 uncharged hydrophobic residues including ten consecutive alanine residues, may act as an α -helical stop-transfer sequence to anchor the 70 kDa protein to the outer membrane (Hurt *et al.*, 1985b). The difference in the ultimate localization of cytochrome *c*₁ and the 70 kDa protein may be due to the closer proximity of the matrix-targeting and the membrane-anchoring sequences in the latter. The intermembranous space protein cytochrome *c* is synthesized as an apoprotein without a cleavable transit sequence, so that more than one import mechanism may operate.

The various import pathways of proteins into mitochondria are summarized in Fig. 2(b).

Chloroplast transit sequences

The *N*-terminal section of the initially-translated small subunit of ribulose 1,5-bisphosphate carboxylase ('RuBisCo') will cause import of the bacterial protein neomycin phosphotransferase into chloroplasts (van der Broeck *et al.*, 1985). Transit sequences in chloroplast precursors are also rather long (36–110 residues) and contain sufficient information to direct proteins to particular chloroplast compartments. Thus the transit peptide for the stromal protein ferredoxin has been shown to direct plastocyanin (minus its own transit sequence) away from its correct location in the thylakoid lumen to the stroma (Smeekens *et al.*, 1986). Curiously, a chloroplast transit sequence has been reported to transport dihydrofolate reductase into the yeast mitochondrial matrix, albeit less efficiently than the transit sequence of mitochondrial cytochrome oxidase subunit IV (Hurt *et al.*, 1986), suggesting that the import mechanisms operating in these two organelles are similar, and may have diverged relatively recently during evolution.

Post-translational movement into mitochondria and chloroplasts

Translation of cytochrome *c*, porin and several matrix-limited proteins occurs predominantly on free ribosomes, indicating that uptake into mitochondria is post-translational; uptake can be demonstrated *in vitro* in the absence of protein translation. The precursors enter mitochondria rapidly, with half-lives of about 1 min, while in the absence of import some precursors degrade fairly rapidly in the cytosol (Raymond & Shore, 1981).

Import of proteins into the mitochondrial matrix, in

contrast to import across or assembly into the outer mitochondrial membrane, requires an electrochemical potential across the inner membrane (Nelson & Schatz, 1979). An energized inner membrane is not required for input of cytochrome *c* into the intermembranous space, but cytochrome *b*₅ is imported to be loosely attached to the inner membrane under the same conditions as the matrix proteins. Precisely how the electrochemical gradient is utilized is not clear, but possibly it labilizes lipid bilayers to induce formation of inverted micelles that conduct transmembrane movement, or alternatively acts to electrophorese the transit peptide across the membrane. It should be noted however that the movement of proteins with respect to charge field is in the opposite direction in mitochondria (from positive to negative) to that in bacteria (from negative to positive).

mRNA encoding for the small subunit of ribulose-bisphosphate carboxylase is associated with free polyosomes, consistent with the virtual absence of ribosomes associated with chloroplasts as viewed under the electron microscope (Dobberstein *et al.*, 1977). Post-translational uptake into chloroplasts has been demonstrated for a number of chloroplast precursor proteins made *in vitro* after addition of protein synthesis inhibitors (Chua & Schmidt, 1979). In chloroplasts, ATP rather than an electrochemical gradient supplies the energy for transport of thylakoid and stromal proteins (Grossman *et al.*, 1980).

Structure of transit sequences

Mitochondrial transit peptides also have no pronounced sequence homology and are extremely variable in length, but are generally highly basic, contain no acidic residues and have a high percentage of leucine, serine and arginine residues (Table 1). The regular spacing of positive charges along the primary sequence suggested to von Heijne (1986) that transit sequences adopt a common secondary structure of a helix with a high hydrophobic moment. Synthetic peptides representing 15, 25 or 33 residues of the *N*-terminus of the precursor of subunit IV of yeast cytochrome oxidase are found to become partially helical in detergent micelles (Roise *et al.*, 1986). Schleyer & Neupert (1985) have recently shown that in *Neurospora* the energy-dependent step is penetration by the *N*-terminus across translocation sites where the inner and outer membranes come into contact into the matrix. Import of the rest of the protein is completed in an energy-independent step. Import of dihydrofolate reductase fused to a transit sequence is prevented by the presence of the enzyme's substrate, possibly by preventing an unfolding change at the mitochondrial membrane (Eilers & Schatz, 1986).

Chloroplast transit peptides are more homologous than mitochondrial transit sequences, contain limited regions of hydrophobic residues, many hydroxyl residues (threonine and serine), fewer basic residues and no acidic residues (Table 1). Three blocks of homology have been identified in the transit sequences of ribulosebisphosphate carboxylase, the light-harvesting *a/b* protein of the thylakoid membrane and ferredoxin (Karlin-Neumann & Tobin, 1986); these are designated for the wheat ribulosebisphosphate carboxylase sequence in Table 1. Domain I is quite hydrophobic, but contains several serine and threonine residues. Domain II exhibits evolutionary conservation in the sequence Gly-Leu-Lys, and is just on the *C*-terminal side of where the first

cleavage takes place between glutamine and glycine; this domain may be part of the sequence that interacts with an envelop receptor. The third domain contains a region of homology close to the second cleavage site (on either side of the methionine; see Table 1). Chou and Fasman predictions suggest that chloroplast transit sequences may be α -helical (Schmidt *et al.*, 1979), while other calculations suggest they are disposed towards β -structure (Pongor & Szalay, 1985).

Cleavage of transit peptides

The transit peptides of many imported mitochondrial or chloroplast precursors are cleaved after import by peptidases which are highly specific for the precursor (Hay *et al.*, 1984). The transit peptide of the small subunit in ribulosebisphosphate carboxylase is proteolytically removed in two steps (Robinson & Ellis, 1984) by soluble proteases during or immediately after import, prior to assembly with the endogenous large subunit, and the precursor of plastocyanin is proteolytically processed in two steps, once in the stroma after crossing the chloroplast envelope to yield an intermediate, and finally in the thylakoid lumen (Smeekens *et al.*, 1986).

Cleavage of mitochondrial precursors occurs by a chelator-sensitive protease in the matrix (Teintze *et al.*, 1982). Some mitochondrial precursors are also cleaved to their mature forms in two steps. Yeast cytochrome b_2 , cytochrome c_1 and cytochrome c peroxidase transit sequences are cleaved first by the matrix protease, then a second cleavage is performed by a chelator-insensitive protease on the outer surface of the inner membrane (Hay *et al.*, 1984). Cytochrome b_2 and cytochrome c peroxidase are released as soluble components of the intermembrane space, whereas cytochrome c_1 remains bound to the outer surface of the inner membrane.

Components that aid the import of mitochondrial and chloroplast precursors

A proteinaceous 40 kDa component of yeast cytosol has been found essential for import of the β -subunit of the mitochondrial ATPase and cytochrome b_2 (Ohta & Schatz, 1984). Moreover, a ribonucleoprotein has been reported to be required for uptake of six different mitochondrial precursors (Firgaira *et al.*, 1984). There is evidence that protease-sensitive receptors associated with the outer membrane of mitochondria are involved in the uptake of proteins other than those that remain in the outer membrane. Specific, saturable binding of apo-cytochrome c to mitochondria has been measured (Korb & Neupert, 1978), and a synthetic peptide corresponding to the transit sequence of ornithine carbamoyltransferase inhibits import of several other precursors of both matrix and inner membrane proteins in a dose-dependent manner (Gillespie *et al.*, 1985). Isolated envelope membranes bind precursors of chloroplast proteins, while both binding and import is prevented by pretreating isolated chloroplasts with proteases, which presumably leads to destruction of receptors (Cline *et al.*, 1984).

Synthetic transit peptides

Synthetic peptides resembling the transit sequences of subunit IV of yeast cytochrome oxidase spontaneously insert into phospholipid monolayers, with a large increase in surface pressure likely to be due to insertion of their hydrophobic regions into the monolayer (Roise

et al., 1986). The peptides also disrupt unilamellar liposomes, a lytic action which is enhanced by a transmembrane potential, negative inside.

The results indicate that initial local disruption of membranes by transit peptides in their precursors may aid their translocation. The synthetic peptides are α -helical in SDS solution. We have recently synthesized a peptide containing residues 1–23 of the wheat ribulosebisphosphate carboxylase transit sequence; unlike many signal peptides it is readily water-soluble and studies are in progress on its physical and biological properties.

SUMMARY

Recent advances have led to considerable convergence in ideas of the way topogenic sequences act to translocate proteins across various intracellular membranes (Table 2). Whereas co-translational translocation and processing were previously considered the norm at the endoplasmic reticulum membrane, several instances of post-translational translocation into endoplasmic reticulum microsomes *in vitro* have now been described. However, it must be noted that post-translational translocation *in vitro* is much less efficient than when endoplasmic reticulum membranes are present during translation, and it is possible that in the intact cell translocation occurs during translation. Movement of proteins into chloroplasts and mitochondria occurs after translation. When translocation is post-translational, proteins may perhaps traverse the membrane as folded domains, and the conformational effects of topogenic sequences on these domains may be as envisaged in Wickner's 'membrane-trigger hypothesis'.

Both signal and transit sequences possess amphipathic structures which are capable of interacting with phospholipid bilayers, and these interactions may disturb the bilayer sufficiently to allow entry of the following domains of protein. There is increasing evidence that GTP is required to bind ribosomes and their associated nascent chains to the endoplasmic reticulum membrane. Precisely how the cell's energy is applied to achieve translocation is not clear, but one possibility at the endoplasmic reticulum is that a GTP-hydrolysing transducing mechanism may exist to couple signal sequence receptor binding to movement of the nascent chain across the membrane. Electrochemical gradients are required for protein movement to the mitochondrial inner membrane and across the bacterial inner membrane. Cytoplasmic factors such as SRP, the *secA* gene product or a 40 kDa protein (for mitochondrial precursors) may act by binding to topogenic sequences and preventing precursor proteins as they are translated from folding into forms which cannot be translocated. Specificity in the cell may be achieved both by targetting interactions between these cytoplasmic factors and their receptors located in target membranes, and also by specific binding of the topogenic sequences to specific proteins integrated into the target membranes. Possible candidates for the latter are the protein of microsomal membranes that reacts with a photoreactive signal peptide to give a 45 kDa complex (Fig. 1), the *secY* gene product of the bacterial inner membrane, and receptors on the outer membranes of chloroplasts and mitochondria. Whether these aid translocation as well as recognition is not clear.

Table 2. Features of protein movement to or across various membranes

Membrane	Disposition	Topogenic sequence	Example	Recognition			Translocation		
				Cytoplasmic receptor	Membrane receptor	Translocation coupled	Energy	Translocation coupled	Proteolysis
Endoplasmic reticulum	Cytoplasmic Transmembrane	Yes	Cytochrome <i>b₂</i>	No	No	No	No	No	None
		Yes	Vesicular stomatitis glycoprotein G	SRP	Yes	Yes	ATP/GTP	?	Sometimes
Bacterial	Secreted Cytoplasmic	Yes	Prolactin	SRP	43 kDa <i>secY</i>	Yes	ATP/GTP	?	Generally
		Yes	LacY	<i>secA</i>	Yes	Yes	Chemical membrane potential	No	Varies
Mitochondria	Periplasmic/outer	Yes	MalE/LamB	<i>secA</i>	<i>secY</i>	Yes	Chemical membrane potential	No	Yes
		Yes	70 kDa protein	No	No	No	No	No	No
	Intermembranous space	Yes	Cytochrome <i>c₁</i>	40 kDa ribonucleoprotein	Yes	No	Electrochemical gradient	No	Yes (× 2)
		Yes	Cytochrome <i>c</i> oxidase	40 kDa ribonucleoprotein	Yes	No	Electrochemical gradient	No	Yes
Chloroplasts	Stroma	Yes	Small subunit of RuBisCo	?	Yes	No	ATP	No	Yes (× 2)
	Thylakoid	Yes	Plastocyanin	?	Yes	No	ATP	No	Yes (× 2)

By application of the rapidly advancing techniques of gene fusion and site-specific mutagenesis, the structural parameters of topogenic sequences and the way they direct the various events involved in targetting, translocation and processing will become more closely delineated. In particular, the influence of folding or unfolding of the polypeptide chain upon translocation could be approached by changes calculated to have dramatic effects on flexibility and secondary structure. In addition, new approaches to the identification and isolation of components that input energy into translocation will provide informative results.

We gratefully acknowledge the support of the Wellcome Trust.

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