MECHANISM OF PROTEIN TRANSLOCATION ACROSS THE ENDOPLASMIC RETICULUM MEMBRANE

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INTRODUCTION

In this review we attempt a timely survey of issues concerning protein translocation across the membrane of the endoplasmic reticulum of eukaryotic cells. We focus on recent developments, open questions and current controversies. Due to limited space, this review cannot be and is not

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intended to be comprehensive. Where appropriate, reference to more detailed reviews is given in the text.

Eukaryotic cells contain a multiplicity of membrane-delimited compartments. The selective localization of particular proteins provides the basis for each of these compartments to serve various specialized functions. Thus, for example, the mitochondrion is the exclusive residence of enzymes involved in oxidative phosphorylation; similarly, oxidative detoxification takes place exclusively in the endoplasmic reticulum (ER). The proteins that compose, and are contained within, particular membrane systems are kept there by the impermeability of the lipid bilayer to diffusion of proteins across membranes. How then is compartmentalization of newly synthesized proteins achieved, in view of the fact that the cytosol is the common site of synthesis for the majority of proteins, though they are destined for distinct subcellular locations? The term intracellular protein topogenesis has been coined (Blobel 1980) to describe the specialized mechanisms by which newly synthesized proteins selectively overcome the permeability barrier of specific intracellular membranes to achieve their correct subcellular localization. This review addresses the question of how proteins that pass through or reside in the intracisternal space are specifically synthesized on membrane-bound ribosomes and translocated into the ER lumen.

As in the study of other protein translocation events (e.g. across mitochondrial membranes) there are two fundamental issues to resolve regarding transport across the ER membrane: (a) How is the target membrane recognized and distinguished from all other membrane systems? (b) Once it has been targeted, how is the polypeptide chain translocated across the lipid bilayer into the lumen of the organelle?

HISTORICAL BACKGROUND

The work of Palade and coworkers on the secretory pathway (reviewed by Palade 1975) focused attention on ribosomes bound to the rough endoplasmic reticulum as the site of synthesis of secretory proteins. The subsequent demonstration of vectorial discharge of puromycin-released polypeptides into the lumen of isolated rough microsomal vesicles (Redman & Sabatini 1966) suggested that a specialized mechanism was responsible for translocation across the ER membrane: Nascent polypeptides emerged into the lumen of the microsomal vesicles concomitant with their synthesis. These results raised the intriguing question of how the cell could distinguish the mRNAs for secretory proteins from those for cytoplasmic or mitochondrial proteins and selectively translate the former on ER-bound ribosomes.

The signal hypothesis (Blobel & Dobberstein 1975) was proposed to account for these phenomena. Over the last 15 years overwhelming evidence has accumulated from a plethora of experimental systems in favor of this model. As it specifically relates to secretory proteins, the essential tenets of an updated version of this hypothesis (for a recent review see Walter et al 1984) are that: (a) the information for localization of newly synthesized proteins into the lumen of the ER is encoded in a discrete segment of the nascent polypeptide, the signal sequence; (b) this signal sequence interacts with a series of receptors, some of them cytoplasmic, others integral to the ER membrane. Some of these receptors function in targeting the chain to the ER membrane, others function in its actual translocation across that membrane. These latter receptors, together with associated proteins in the ER membrane, constitute the "translocon," a postulated engine able to drive signal sequence–bearing chains across the ER membrane through a proteinaceous pore or channel.

More recently, the concepts of the signal hypothesis have been expanded to describe a general framework for intracellular protein topogenesis (Blobel 1980). According to this model, "topogenic sequences" within discrete segments of targeted proteins are decoded by specific receptors, either during (cotranslational) or shortly after (posttranslational) their biosynthesis. The specificity of such signal sequence–receptor interactions targets the proteins to the correct intracellular membranes where they are fed into translocons that move them across the hydrophobic core of the lipid bilayer. Similarly, it has been proposed that another class of topogenic sequences—termed stop-transfer sequences—interacts with the translocon to arrest further transport and thereby achieve an asymmetric transmembrane orientation of integral membrane proteins. Thus many of the concepts developed in this review for soluble ectoplasmic proteins are directly applicable to the problem of integration of transmembrane proteins. Recent developments reviewed below suggest that translocons in different intracellular membrane systems may function more similarly than previously thought.

MECHANISM OF TARGETING

With the availability of in vitro systems that faithfully reproduce the translocation of nascent proteins [secretory proteins (Blobel & Dobberstein 1975), lysosomal proteins (Erickson et al 1983), and certain classes of integral membrane proteins (Katz et al 1977)], it became feasible to investigate the molecular requirements for protein translocation across the ER membrane. So far, two components, the signal recognition particle

(SRP) and the SRP receptor, have been purified and shown to function in the targeting events preceding the actual translocation event.

Signal Recognition Particle

SRP is an 11S small cytoplasmic ribonucleoprotein (Walter & Blobel 1982). In our current view, SRP functions as an adapter between the protein synthetic machinery in the cytoplasm and the protein translocation machinery in the ER membrane.

STRUCTURE OF SRP   SRP was first recognized by its ability to restore the translocation activity of salt-extracted microsomes in vitro (Warren & Dobberstein 1978). It was purified to homogeneity from a salt extract of canine pancreatic microsomal vesicles using this activity as an assay (Walter & Blobel 1980). SRP consists of a small (300 nucleotide) 7SL RNA (Walter & Blobel 1982) and six nonidentical polypeptide chains organized into four SRP proteins. These proteins are two monomers, a 19-kDa polypeptide and a 54-kDa polypeptide, and two heterodimers, one composed of a 9-kDa and a 14-kDa polypeptide, and the other comprised of a 68-kDa and a 72-kDa polypeptide (Siegel & Walter 1985). When SRP is disassembled under nondenaturing conditions, the RNA and the protein fractions are inactive by themselves, but together they can readily be reconstituted into an active particle (Walter & Blobel 1983; Siegel & Walter 1985).

Recent studies revealed that different assayable functions of SRP in the targeting process can be assigned to specific structural domains of the particle. These separable functions include the recognition of signal sequences and the ability of SRP to arrest specifically the translation of nascent signal sequence–bearing proteins (Siegel & Walter 1986b). These domains are schematically indicated in Figure 1 superimposed on the secondary structure of 7SL RNA. This model is supported by recent evidence demonstrating that SRP is a rod-shaped, elongated structure (Andrews et al 1985) and that the RNAs—visualized directly by electron spectroscopic imaging—span the entire length of the particle (D. W. Andrews et al, submitted for publication).

SIGNAL RECOGNITION   Once SRP had been purified to homogeneity it became possible to study its activity in greater detail. Results of experiments testing both the effects of SRP on the translation of secretory proteins *and* its binding properties with various components in the translation-translocation system have led to the model of the SRP cycle shown in Figure 2.

In brief, SRP is thought to bind in a signal-sequence-independent

manner with relatively low affinity to biosynthetically inactive ribosomes (Figure 2a, b) (Walter et al 1981). Upon emergence of a signal sequence as part of the nascent polypeptide chain, the affinity of SRP for the ribosome increases (Figure 2c); in the case of preprolactin synthesized on wheat germ ribosomes this increase amounts to three to four orders of magnitude. The SRP-ribosome-nascent chain complex is then targeted to the membrane of the ER via a direct interaction of SRP with the SRP receptor (Walter & Blobel 1981b), an integral membrane protein that is restricted in its subcellular localization to this membrane system (Hortsch et al 1985). At this point SRP and the SRP receptor detach from the ribosome and can reenter the cycle, i.e. both molecules are thought to act catalytically in the targeting process. The ribosome-nascent chain complex engages in a functional ribosome membrane junction, and the translocation of the nascent polypeptide proceeds (see below). (For a more detailed description of the SRP cycle see Walter et al 1984.)

ELONGATION ARREST   When SRP is included in in vitro translation systems in the absence of microsomal membranes, it blocks protein synthesis concomitant with the increase in its affinity for the ribosome just after the signal peptide becomes exposed outside the large ribosomal subunit (Walter & Blobel 1981b; Meyer et al 1982a). In some cases a discretely sized protein fragment that corresponds to the elongation-arrested secretory protein can be detected by gel electrophoresis; in other cases the arrested forms appear as a broader smear on gels, which indicates that SRP can recognize signal sequences and arrest elongation within a certain range of chain lengths. It is also observed that some nascent polypeptides are arrested, while others transiently pause in chain growth (P. Walter, unpublished results). Therefore, in these latter cases arrest is often difficult to detect (Meyer 1985). Interestingly, while elongation arrest has been demonstrated as a kinetic delay of elongation in translation systems reconstituted from mammalian components (K. Matlack & P. Walter, unpublished results), the same effect is more pronounced (as a strict blockage of elongation) when signal-bearing proteins are translated in a heterologous wheat germ system. Thus while the general phenomenon of arrested elongation is ubiquitous, different in vitro systems reflect it to a different degree. Therefore it remains to be established whether SRP acts in vivo as a strict "on-off" switch or functions as a more graded rate-controlling factor.

Two distinct biochemical approaches were employed to map the elongation-arrest function to a separate and separable domain of SRP. One functional domain was shown to consist of the 9/14-kDa SRP proteins and those 7SL RNA sequences that are homologous to repetitive Alu DNA (see Figure 1, left). One experimental approach employed single omission

experiments in which SRPs were reconstituted from fractionated and
purified protein and RNA components (Siegel & Walter 1985). A second
approach involved the preparation of a subparticle obtained after nucle-
olytic dissection of SRP (Siegel & Walter 1986). These perturbed SRPs
lacking the elongation-arrest domain are still active in signal recognition
and targeting; therefore, elongation arrest *cannot* be a prerequisite for
protein translocation across the membrane. In the absence of elongation
arrest, however, most signal-bearing nascent proteins lose their ability to

(a)

(b)
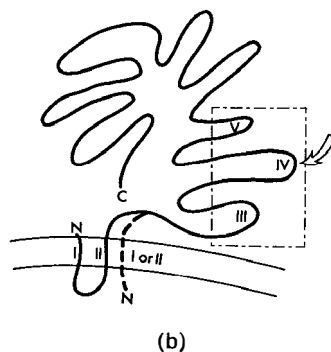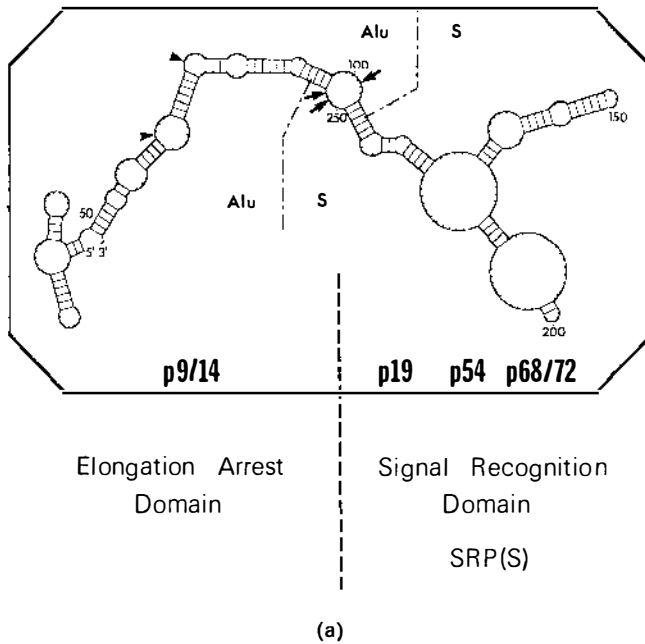
be translocated if elongation proceeds beyond a critical point in the absence of membranes. Thus elongation arrest seems to maintain the nascent chain in a translocation-competent state by preventing (or delaying) its further elongation into the cytoplasmic space and thereby adds to the fidelity of the reaction. The particular length range in which a nascent protein remains translocation competent may vary for different proteins (see below).

Since SRP contains an RNA as a structural component, it is tempting to speculate that this RNA engages in base-pairing interactions with other nucleic acids during the SRP's functional cycle. The RNA components in the translational apparatus are likely candidates for participants in such interactions (Walter & Blobel 1982; Zwieb 1985). However, there is at present no direct evidence for such interactions. A possible mechanism for elongation arrest could involve the binding of 7SL RNA to the A-site on the ribosome, thus preventing the next amino acyl tRNA from binding. Indeed, the secondary structure of 7SL RNA in the elongation-arrest

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Figure 1   Domain structure of SRP (*left*) and the SRP receptor (*right*). (*a*) (From Siegel & Walter 1986a): SRP is composed of two separable domains. A possible phylogenetically conserved secondary structure for 7SL RNA is shown (Siegel & Walter 1986a). Similar secondary structures have been proposed by Gundelfinger et al (1984), E. Ullu (personal communication), and Zwieb (1985). Connecting lines between the RNA strands indicate base pairs; G-U pairs are included. (For an extensive description of SRP structure see Siegel & Walter 1986b.) Micrococcal nuclease cleaves the particle at the point indicated by arrows, removing the elongation-arresting domain. Additional cuts mapped by Gundelfinger et al (1983) are indicated by arrowheads. The elongation-arresting domain includes both ends of the RNA (labeled 5' and 3') and is comprised of sequences that are homologous to the repetitive Alu DNA sequence family. Evolutionary considerations suggest that 7SL RNA is the parent molecule for repetitive Alu DNA (Ullu & Tschudi 1985). The thin dashed lines indicate the boundaries of homology between 7SL RNA and an Alu consensus sequence. The elongation-arresting domain also contains the 9/14-kDa SRP protein. The other domain, termed SRP(S), retains signal recognition and translocation promoting function and is comprised of the middle portion of 7SL RNA (the S-segment) and the remaining three SRP proteins. As mentioned in the text, the 54-kDa SRP protein can be selectively cross-linked to signal peptides and may therefore provide the signal binding pocket. (*b*) (From Lauffer et al 1985): A model of the disposition of the SRP receptor α-subunit in the membrane of the ER is shown. Putative structural and functional features as deduced from the primary sequence (Lauffer et al 1985) are indicated. Regions I and II are putative membrane-spanning regions; whether both of them or either one alone functions as the membrane anchor of the receptor or if additional hydrophobic regions are contributed by the β-subunit is presently not known. Regions III–V contain the charge clusters described in the text. The boxed domain contains regions strongly resembling RNA binding proteins; their presence suggests that the SRP–SRP receptor interaction may include binding of 7SL RNA to this domain. The arrow indicates the position of the protease-sensitive site. Cleavage of the receptor at this position results in the release of the 52-kDa cytoplasmic fragment. This fragment does not have two properties of the intact receptor: the binding affinity for SRP and the ability to release elongation arrest (Lauffer et al 1985; Gilmore et al 1982a).
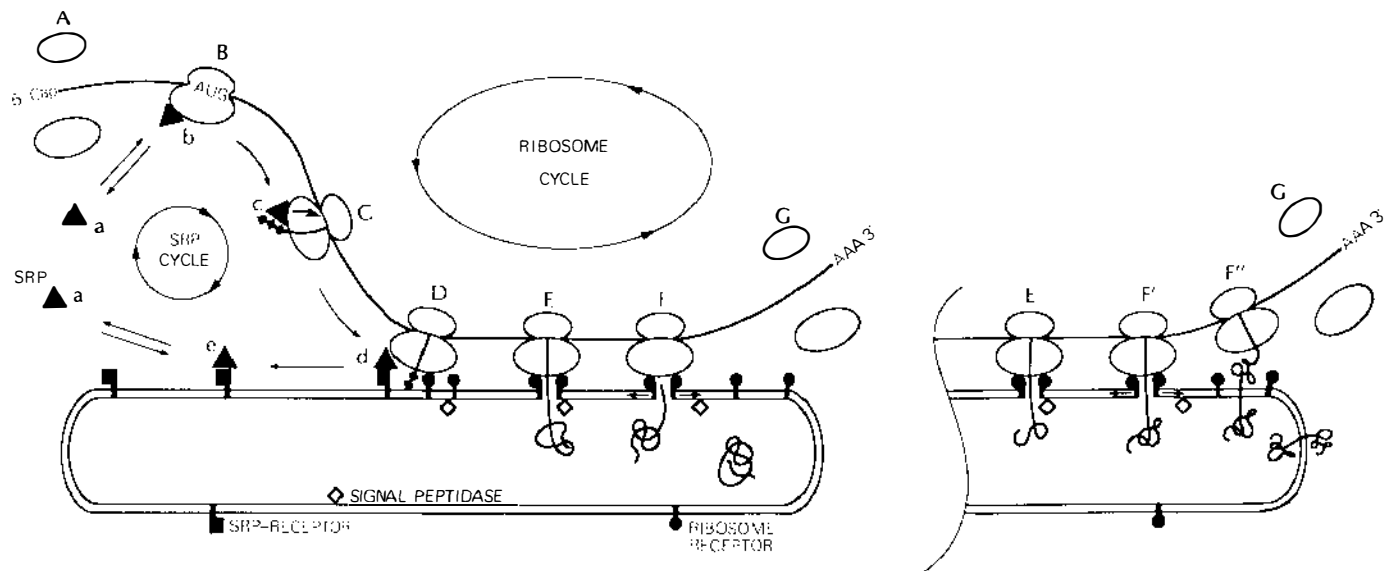
Figure 2   Model (from Walter et al 1984) for protein translocation across the ER membrane for soluble intracisternal proteins (*left*) and integral membrane proteins that possess a structural domain on the intracisternal face of the membrane (*right*). The key features of the model are outlined in the text. (For a more extensive description see Walter et al 1984.)

domain of SRP resembles that of a tRNA that is missing the anticodon stem. In addition, the physical dimensions of SRP would easily allow the particle to bridge the distance between the nascent chain exit site on the ribosome (where the signal sequence emerges) and the peptidyl transferase activity known to be located between the two ribosomal subunits (Andrews et al 1985).

Signal Sequences

What constitutes the essential features of a signal sequence and how such sequences are recognized by SRP remain unsolved problems. Signal sequences show no recognizable primary sequence homology, and a recent compilation shows that sequence variation can be rather extreme (von Heijne 1985). Yet studies on a variety of systems both in vivo and in vitro demonstrate conservation of signal sequence function over the widest evolutionary distances (Muller et al 1982). As a consequence we are still not able to predict with confidence which regions in proteins might function as internal signal sequences. Nevertheless, internal signal sequences have been demonstrated unequivocally (Bos et al 1984). Moreover, cleavage by signal peptidase is not required for translocation (Palmiter et al 1978).

One of the few characteristic features of signal sequences is a variable stretch of hydrophobic amino acids in the core of the sequence. Point mutations in the hydrophobic core in bacterial signal sequences have been shown to abolish function (Lee & Beckwith 1986, this volume). Based on the hydrophobicity of these regions and on evidence from biophysical studies with synthetic signal peptides (reviewed by Briggs & Gierasch 1986), it has been suggested that these sequences act as amphiphiles that are integrated into and possibly perturb lipid bilayers. There is, however, still no evidence that the general mechanism for translocation involves a direct interaction of signal sequences with the hydrophobic core of the lipid bilayer. Indeed, several lines of evidence suggest direct interactions of signal sequences with *proteins*.

The clearest evidence for such interactions involve SRP. Since SRP is a soluble ribonucleoprotein, its interactions with signal sequences can be studied in the absence of membranes by measuring binding or by observing the SRP-mediated modulation of protein synthesis. For example, when signal sequences that are rich in leucine are translated in the presence of the amino acid analog β-hydroxy-leucine, SRP signal recognition is abolished (Walter et al 1981; Walter & Blobel 1981b). This demonstrates that SRP directly recognizes features in the nascent chain. Moreover, the finding conclusively rules out the possibility that sequences in the mRNA alone are responsible for the observed effect. (After the discovery of an RNA component in SRP the latter notion was considered attractive

because of the possibility of recognition via putative base-pairing inter-
actions.) Direct proof of an SRP–signal sequence interaction was recently
provided by cross-linking experiments. Two groups independently showed
that a photoactivable cross-linking reagent was selectively incorporated
into the amino-terminal region of the signal peptide for nascent prepro-
lactin. Each group found that the signal peptide is in *direct* contact with
the 54-kDa SRP protein (Kurzchalia et al 1986; Krieg et al 1986).

SRP Receptor

Using the same in vitro protein translocation assays that led to the puri-
fication of SRP, two distinct approaches were taken to identify the cor-
responding *membrane* components involved in targeting of signal
sequence–bearing nascent chains to the ER membrane. These approaches
eventually led to the discovery and purification of the SRP receptor, the
first membrane protein proven to play a vital role in this process.

One of these approaches was based on the early observation that pro-
teolysis of microsomal membranes completely abolishes their protein
translocation activity but that, most importantly, the activity can be
restored by addition to an extract prepared by limited proteolysis of the
original microsomal membrane fraction (Walter et al 1979; Meyer &
Dobberstein 1980a). This proteolytic dissection and functional recon-
stitution provided the assay for the purification of the protease-solubilized
component. The activity was purified as a basic 52-kDa protein (apparent
mobility on SDS PAGE is 60 kDa) (Meyer & Dobberstein 1980b), which
was subsequently demonstrated (by immunological techniques) to be a
proteolytic fragment derived from a 69-kDa integral membrane protein
(apparent mobility 72 kDa) restricted in its subcellular localization to the
endoplasmic reticulum (Meyer et al 1982b).

The second approach took advantage of the observations that, when
assayed in the absence of microsomal membranes, SRP causes a site-
specific elongation arrest in the synthesis of presecretory proteins and that
microsomal membranes contain an activity that releases the elongation
arrest. Based on these observations, the elongation-arrest-releasing activity
was predicted to reside in a membrane protein termed the SRP receptor
(Walter & Blobel 1981b) [subsequently named the docking protein (Meyer
et al 1982a)]. Fractionation of a detergent extract of microsomal mem-
branes employing affinity chromatography on SRP-Sepharose as a key step
allowed purification of the SRP receptor. The purified fraction contained a
predominant 69-kDa membrane protein and the arrest-releasing activity.
Using both immunological and peptide-mapping techniques, the SRP
receptor was shown to be identical to the membrane protein identified via
the proteolytic dissection methods described above (Gilmore et al 1982a,b).

Recently, the primary structure of the 69-kDa SRP receptor protein was determined from its cognate cloned cDNA, and its relationship to the cytoplasmic SRP receptor fragment was determined (Lauffer et al 1985). This fragment was shown to begin with residue 152 of the intact protein. Thus, it is sequences within the 151 amino acids at the amino terminal that anchor the SRP receptor in the lipid bilayer. Two distinctly hydrophobic regions have been identified that constitute putative α-helical trans-membrane segments. Since either of these segments would position a positively charged amino acid in the hydrophobic core of the lipid bilayer, the receptor probably interacts with other integral membrane proteins that neutralize these charges. Recent evidence suggests the existence of proteins that can be copurified with the 69-kDa SRP receptor protein or isolated by affinity techniques. In particular, an ER membrane protein with an apparent molecular weight of 30 kDa was found by a variety of techniques to be tightly associated with the 69-kDa protein (Tajima et al 1986). Thus the SRP receptor appears to be a hetero-dimeric protein that in addition to the 69-kDa polypeptide (the SRP receptor α-subunit) contains a second 30-kDa subunit (β-subunit). Carboxy-terminal to the putative trans-membrane regions in the α-subunit is an unusually hydrophilic domain. In particular, unusually large clusters of charged amino acids are found surrounding the site of proteolytic cleavage that severs the 52-kDa cyto-plasmic domain (see Figure 1, *right*). This domain of the SRP receptor strongly resembles nucleic acid binding proteins, which suggests that the receptor may transiently interact directly with the 7SL RNA in SRP and that the SRP–SRP receptor affinity could be mediated, at least in part, by a protein–nucleic acid interaction.

The SRP receptor is unlikely to be part of the translocon itself, because the receptor is present in the ER membrane in substoichiometric amounts with respect to membrane-bound ribosomes. Thus it was suggested that the SRP receptor functions "catalytically" and is recycled once correct targeting of the ribosome has been achieved (Gilmore & Blobel 1983). There is also evidence for an additional activity that is distinct from SRP and the SRP receptor and may interact with the targeted signal sequence and act as a secondary signal receptor(s) in the ER membrane (Gilmore & Blobel 1985; Prehn et al 1980). However, a protein serving this function has not yet been identified.

MECHANISM OF TRANSLOCATION

Machinery

Cell-free systems provided a detailed molecular description of the targeting machinery, but have yet to allow insights into the molecular details of the

translocation process. In part this difficulty results from the apparent obligate coupling of translocation and translation: Transport across the ER membrane takes place cotranslationally; completed precursors are not detectable in vivo in the cytoplasm. In cell-free systems translocation proceeds only during a limited time and under the fastidious conditions required for the synthesis of the very molecule whose translocation is being studied. As a result, although several specific polypeptides have been implicated as functional components of the translocon, the direct role of any of these proteins remains to be demonstrated. For example, two integral membrane proteins, termed ribophorins, have been suggested to act as ribosome receptors (Kreibich et al 1978); the recent purification of signal peptidase, a relatively abundant complex of six polypeptides, suggests that these proteins are involved in other functions besides signal cleavage (Evans et al 1986).

Translocation Substrates

Although we know little about the actual machinery involved, insight into certain aspects of the mechanism of translocation has recently been obtained by approaches involving manipulation of the translocation substrates. For example, expression of engineered cDNAs encoding fusion proteins in transcription-linked translation systems demonstrated that a signal sequence was sufficient to direct translocation of normally cytoplasmic globin, both in vitro (Lingappa et al 1984) and in vivo (K. Simon et al, submitted for publication). Thus, the specific information for translocation was contained within the signal sequence and not the "passenger" protein.

A more complex version of these experiments raised interesting questions as to the mechanism of translocation (Perara & Lingappa 1985). The DNA sequence coding for globin, normally a cytosolic protein, was fused with the 5′ end of the DNA sequence for preprolactin, a secretory protein that has an amino-terminal signal sequence. This fusion protein thus contained the preprolactin signal sequence at an internal position, 117 amino acids from the initiator methionine. When expressed in a transcription-linked translation system, this internal signal sequence was not only cleaved by signal peptidase, but directed the translocation of both flanking protein domains. Surprisingly, carbonate extraction demonstrated that neither the globin domain with the signal sequence attached at its carboxy terminus nor the prolactin domain were integrated into the membrane. Instead, both resided in the vesicle lumen either free or bound to proteins. This result suggests that signal sequences are not buried in the bilayer directly but perform their function by interacting with a protein-

aceous machinery in the membrane. Moreover, translocation of the globin domain by a subsequently emerging signal sequence suggests that the energy used for the globin domain's synthesis is not required for its translocation. Thus the commonly observed coupling of translocation and translation may not be an obligate requirement for transport across the ER membrane.

The notion that the translocation machinery can function independently of protein synthesis has now received direct support from different experimental systems.

Posttranslational Translocation in Yeast

Recently, in vitro translation-translocation systems from the yeast *Saccharomyces cerevisiae* have been established (Hansen et al 1986; Waters & Blobel 1986; Rothblatt & Meyer 1986). The precursor to the yeast pheromone α-factor has been used as a model secretory protein. Contrary to all expectations, this precursor, an ~ 18.5 kDa protein, is translocated across yeast ER membranes posttranslationally, i.e. after it has been completely synthesized and has been released from ribosomes. Prepro-α-factor has no particularly hydrophobic or amphipathic stretches in its primary sequence (other than a typical signal sequence), making it unlikely that its posttranslational translocation is due to some passive partitioning of the protein across the lipid bilayer. Furthermore, the posttranslational translocation reaction is ATP-dependent and requires protein elements both in the membrane and the soluble fraction. Whether these protein components are related in any way to the putative yeast SRP and SRP receptor analogs remains to be established by biochemical analysis. It is clear from these data, however, that translocation of prepro-α-factor does not require coupling to protein synthesis. Therefore, the translocon can, in principle, accept its substrate posttranslationally and in the absence of the ribosome.

It should be kept in mind that the posttranslational translocation of prepro-α-factor was observed in vitro in a system artificially depleted of ER membranes during synthesis. This finding does not prove that prepro-α-factor ever crosses the ER membrane posttranslationally in vivo, where ER membranes are always present during translation. Rather, the actual degree of coupling of translocation and protein synthesis will depend on the relative rates of the respective processes. If targeting and translocation are fast with respect to protein elongation, a strictly vectorial cotranslational translocation mode will result, as appears to be the rule in mammalian cells in vivo (Bergman & Kuehl 1979; Glabe et al 1980).

Posttranslational Translocation of Genetically Engineered Substrates

Similar findings also emerged from the use of engineered clones in mammalian cell-free translation systems (Perara et al 1986; Mueckler & Lodish 1986). Using a procedure that generates a truncated mRNA lacking a termination codon, secretory polypeptide chains could be synthesized and presented to membranes in the absence of further chain elongation while still held by the ribosome that effects their synthesis. It was demonstrated that such chains could be translocated and that nucleotide triphosphates were required as the energy source for this process. In contrast to the situation in the yeast system described above, in most of these cases translocation could be abolished by releasing the nascent chain from the ribosome by artificial termination with the amino acyl tRNA analog puromycin. As expected, translocation was abolished by deletion of the coding region for the signal sequence. In some cases, however, it was also found that some short chains could translocate in a ribosome-independent condition analogous to that found for prepro-α-factor in the yeast system (E. Perara & V. R. Lingappa, submitted for publication). Thus it appears that, at least for the proteins investigated, polypeptide chain growth proceeds through stages in which translocation competence is a property of the chain itself or is maintained by interaction with the ribosome (see Figure 3).

These results show cotranslational translocation in a new light: The role of the membrane-bound ribosome is not to extrude or push the chain through the bilayer as suggested by some observers (Wickner & Lodish 1985). Rather, translocation is catalyzed by an energy-consuming protein engine in the ER membrane, and the ribosome acts, in most but not all cases, as a ligand that maintains the translocation competence of the nascent chain.

CONCEPTS AND CONTROVERSIES

We have surveyed the development of ideas on the problem of translocation of newly synthesized proteins across the ER membrane. Initially, attention was focused on the coupling of translocation to translation, a feature unique to translocation across the ER membrane. This has given way to the realization that obligate coupling to translation is not a prerequisite for translocation and that transport across membranes of a variety of organelles may share common features. These include the involvement of a targeting receptor to discriminate among proteins intended for different destinations, a translocon that somehow transports

the targeted protein across the bilayer, and a requirement for energy (derived from hydrolysis of nucleoside triphosphates or from an electrochemical gradient) to drive translocation. The recognition of these steps has resulted from the study of diverse proteins in a variety of organisms and from the study of "artifacts" generated in vitro, i.e. biochemically or genetically altered translocation machinery (Siegel & Walter 1986b) and substrates (Perara & Lingappa 1985), whose aberrant behavior has provided insight into fundamental details of the targeting and translocation problem. Even as new questions emerge, many old ones (e.g. the molecular nature of the signal sequence–receptor interaction) remain unanswered.

Other questions must now be reformulated. For example, in spite of the recent demonstration that the translocon in the ER membranes can, in principle, accept translocation substrates posttranslationally, translocation most likely occurs cotranslationally in vivo. The observation that most posttranslational translocation across the ER membrane appears to be ribosome dependent in vitro supports this notion. As described earlier, ribosome-independent and ribosome-dependent modes of posttranslational translocation across the ER membrane probably reflect the requirements for maintenance of the "translocation competent state" of the nascent chain (see Figure 3). Loss of translocation competence may be due to folding (aberrant or normal) or oligomerization of the protein, or entanglement of the signal sequence with the rest of the chain such that the resulting structure can no longer functionally interact with either the targeting or translocation machinery. A few proteins (such as yeast prepro-α-factor) retain translocation competence even as free, completed polypeptides. For most proteins, however, translocation competence is restricted to a generally narrow range of chain lengths. This range can be extended if the polypeptide is targeted to the membrane while still attached to the ribosome. However, eventually most proteins reach a point in chain elongation where translocation competence is no longer maintained, even when the protein is associated with the ribosome. One of the roles of the SRP-induced elongation arrest may therefore be to extend the effective range of translocation competence for the nascent polypeptide chains.

Previously, the nascent chain was thought to be vectorially translocated across the membrane as it emerged from the ribosome; the finding of posttranslational translocation raises the possibility that the translocon may be sufficiently pliable to accept (partially) folded domains rather than exclusively linear polypeptide chains. Alternatively, the translocon may effect unfolding of such domains prior to translocation. In either case the molecular environment traversed by the protein as it passes through the bilayer remains to be investigated. The finding that translocation is driven by nucleoside triphosphate hydrolysis is a direct demonstration of a protein
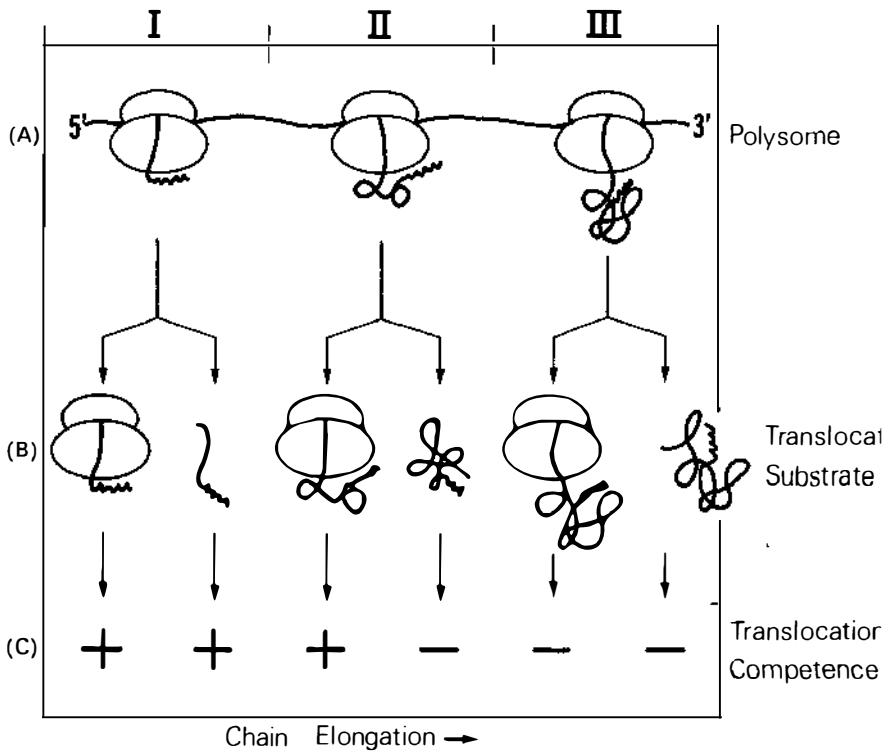
Figure 3 Ribosome dependence of translocation competence. This figure depicts the natural history of the relationship of chain growth (*A*) to translocation competence (*C*). The ribosome dependence of posttranslational translocation was assayed for various lengths of polypeptide synthesized. Progressively shorter polypeptides were synthesized by translating mRNA transcripts in vitro that were progressively truncated at their 3′ end and therefore lacked termination codons (Perara et al 1986; E. Perara & V. R. Lingappa, manuscript in preparation). Ribosomes that have reached the 3′ end of such a truncated mRNA appear unable to release the newly synthesized polypeptide. Release can be artificially achieved by treatment with puromycin. Such translocation substrates, either with or without release from the ribosomes (as indicated in *B*), can be assayed for translocation competence upon presentation to a microsomal membrane preparation in the presence of nucleoside triphosphate to supply energy. In this assay the ribosome dependence or independence of the translocation competence is reflected in the ability or inability of puromycin pretreatment to abolish translocation by releasing the chain from the ribosome (see right arms of branched arrows). (*A*) depicts three ribosomes on a polysome at various stages (I, II, and III) during the synthesis of a hypothetical secretory polypeptide chain. In (*C*) translocatin competence as assayed posttranslationally (see above) is indicated (+). At stage I, the nascent chain is translocation competent, and this competence is independent of the presence of the ribosome, as experimentally demonstrated. As chain growth proceeds, the polypeptide enters stage II where its translocation competence requires the ribosome. Finally, late in chain growth (stage III) the chain is no longer competent to interact with receptors and other proteins involved in translocation. Whether loss of translocation competence in stage III involves a loss of targeting function or loss of a productive interaction with the translocon remains to be determined. It is not known whether SRP is required for posttranslational translocation in either case.

engine in the membrane and rules out a spontaneous process previously suggested (Wickner 1979; Engelman & Steitz 1980). It remains to be established how the energy of hydrolysis is used by the translocon.

Old controversies regarding co- versus posttranslational translocation appear to be resolved. In retrospect it could be concluded that many prokaryotic proteins (targeted to the plasma membrane) do not require ribosomes to maintain their translocation competence. This also appears to be the case for all proteins (so far studied) that are translocated across the peroxisomal membrane and the mitochondrial and chloroplast envelopes. The most challenging problems for future research now include the further fractionation and purification of all the essential, as well as modulatory, components of the targeting and translocation machinery. This should ultimately allow their reconstitution in in vitro systems for the mechanistic analysis of their functions. Finally, our goal must be the understanding of how these components function in vivo. This should include elucidation of the regulatory or homeostatic mechanisms involved in harnessing such a remarkable set of protein machines as the translocons.

Literature Cited

Andrews, D. W., Walter, P., Ottensmeyer, F. P. 1985. *Proc. Natl. Acad. Sci. USA* 82: 785–89

Bergman, L. W., Kuehl, L. M. 1979. *J. Biol. Chem.* 254: 8869–76

Blobel, G. 1980. *Proc. Natl. Acad. Sci. USA* 77: 1496–1500

Blobel, G., Dobberstein, B. 1975. *J. Cell Biol.* 67: 835–51

Bos, T. J., Davis, A. R., Nayak, D. P. 1984. *Proc. Natl. Acad. Sci. USA* 81: 2337–41

Briggs, M. S., Gierasch, L. M. 1986. *Adv. Protein Chem.* 38: In press

Engelman, D. M., Steitz, T. A. 1981. *Cell* 23: 411–22

Erickson, A. H., Walter, P., Blobel, G. 1983. *Biochem. Biophys. Res. Commun.* 115: 275–80

Evans, E., Gilmore, R., Blobel, G. 1986. *Proc. Natl. Acad. Sci. USA* 83: 581–85

Gilmore, R., Blobel, G., Walter, P. 1982a. *J. Cell Biol.* 95: 463–69

Gilmore, R., Blobel, G. 1983. *Cell* 35: 677–85

Gilmore, R., Blobel, G. 1985. *Cell* 42: 497–505

Gilmore, R., Walter, P., Blobel, G. 1982b. *J. Cell Biol.* 95: 470–77

Glabe, C. G., Hanover, J. A., Lennarz, W. J. 1980. *J. Biol. Chem.* 255: 9236–41

Gundelfinger, E. D., Carlo, M. D., Zopf, D., Melli, M. 1984. *EMBO J.* 3: 2325–32

Gundelfinger, E. D., Krause, E., Melli, M., Dobberstein, B. 1983. *Nucleic Acids Res.* 11: 7363–73

Hansen, W. B., Garcia, P. D., Walter, P. 1986. *Cell* 45: 397–406

Hortsch, M., Griffiths, G., Meyer, D. I. 1985. *Eur. J. Cell Biol.* 38: 271–79

Katz, F. N., Rothman, J. E., Lingappa, V. R., Blobel, G., Lodish, H. F. 1977. *Proc. Natl. Acad. Sci. USA* 74: 3278–82

Kreibich, G., Freienstein, C. M., Pereyra, B. N., Ulrich, B. L., Sabatini, D. D. 1978. *J. Cell Biol.* 77: 488–506

Krieg, U., Walter, P., Johnson, A. 1986. *Proc. Natl. Acad. Sci. USA.* In press

Kurzchalia, T. V., Wiedmann, M., Gir-

shovich, A. S., Bochkareva, E. S., Bielka, H., Rapoport, T. A. 1986. *Nature* 320: 634–36

Lauffer, L., Garcia, P. D., Harkins, R. N., Coussens, L., Ullrich, A., Walter, P. 1985. *Nature* 318: 334–38

Lee, C., Beckwith, J. 1986. *Ann. Rev. Cell Biol.* 2: 315–36

Lingappa, V. R., Chaider, J., Yost, C. S., Hedgpeth, J. 1984. *Proc. Natl. Acad. Sci. USA* 81: 456–60

Meyer, D. I. 1985. *EMBO J.* 4: 2031–33

Meyer, D. I., Dobberstein, B. 1980a. *J. Cell Biol.* 87: 498–502.

Meyer, D. I., Dobberstein, B. 1980b. *J. Cell Biol.* 87: 503–8

Meyer, D. I., Krause, E., Dobberstein, B. 1982a. *Nature* 297: 647–50

Meyer, D. I., Louvard, D., Dobberstein, B. 1982b. *J. Cell Biol.* 92: 579–83

Mueckler, M., Lodish, H. F. 1986. *Cell* 44: 629–37

Muller, M., Ibrahimi, I., Chang, C. N., Walter, P., Blobel, G. 1982. *J. Biol. Chem.* 257: 11860–63

Palade, G. 1975. *Science* 189: 347–58

Palmiter, R. D., Gagnon, J., Walsh, K. A. 1978. *Proc. Natl. Acad. Sci. USA* 75: 94–98

Perara, E., Lingappa, V. R. 1985. *J. Cell Biol.* 101: 2292–2301

Perara, E., Rothman, R. E., Lingappa, V. R. 1986. *Science* 232: 348–52

Prehn, S., Nurnberg, P., Rapaport, T. A. 1980. *Eur. J. Biochem.* 107: 185–95

Redman, C. M., Sabatini, D. D. 1966. *Proc. Natl. Acad. Sci. USA* 56: 608–15

Rothblatt, M., Meyer, D. I. 1986. *Cell* 44: 619–28

Siegel, V., Walter, P. 1985. *J. Cell Biol.* 100: 1913–21

Siegel, V., Walter, P. 1986a. *Nature* 320: 81–84

Siegel, V., Walter, P. 1986b. In *Genetic Engineering*, Vol. 8, pp. 179–94, ed. J. K. Setlow. New York: Plenum

Tajima, S., Lauffer, L., Rath, V., Walter, P. 1986. *J. Cell Biol.* 103: In press

Ullu, E., Tschudi, C. 1985. *Nature* 312: 171–72

von Heijne, G. 1985. *J. Mol. Biol.* 184: 99–105

Walter, P., Blobel, G. 1980. *Proc. Natl. Acad. Sci. USA* 77: 7112–16

Walter, P., Blobel, G. 1981a. *J. Cell Biol.* 91: 551–56

Walter, P., Blobel, G. 1981b. *J. Cell Biol.* 91: 557–61

Walter, P., Blobel, G. 1982. *Nature* 299: 691–98

Walter, P., Blobel, G. 1983. *Cell* 34: 525–33

Walter, P., Gilmore, R., Blobel, G. 1984. *Cell* 38: 5–8

Walter, P., Ibrahimi, I., Blobel, G. 1981. *J. Cell Biol.* 91: 545–50

Walter, P., Jackson, R. C., Marcus, M. M., Lingappa, V. R., Blobel, G. 1979. *Proc. Natl. Acad. Sci. USA* 76: 1796–99

Warren, G., Dobberstein, B. 1978. *Nature* 273: 569–71

Waters, G., Blobel, G. 1986. *J. Cell Biol.* 102: 1543–50

Wickner, W. T. 1979. *Ann. Rev. Biochem.* 48: 23–45

Wickner, W. T., Lodish, H. F. 1985. *Science* 230: 400–7

Zwieb, C. 1985. *Nucleic Acids Res.* 13: 6105–24