Site-specific Photocross-linking Reveals That Sec61p and TRAM Contact Different Regions of a Membrane-inserted Signal Sequence*

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Stephen High††‡‡, Bruno Martoglio§§, Dirk Görlich¶, Sören S. L. Andersen¶, Anthony J. Ashford, Angelika Giner, Enno Hartmann§, Siegfried Prehn**‡, Tom A. Rapoport†, Bernhard Dobberstein‡‡, and Josef Brunner‡‡‡

From the European Molecular Biology Laboratory, Postfach 102209, Meyerhofstrasse 1, 6900 Heidelberg, Germany, the §Swiss Federal Institute of Technology (ETH), CH-8092 Zürich, Switzerland, the ¶Max-Delbrück-Center for Molecular Medicine, Robert-Russe-Strasse 10, 1123 Berlin-Buch, Germany, and the **Institute of Biochemistry, Humboldt-University, Hessische-Strasse 3-4, 1040 Berlin, Germany

A chemically charged amber suppressor tRNA was used to introduce the photoactivatable amino acid (Tmd)Phe at a selected position within the signal sequence of the secretory protein preprolactin. This allowed the interactions of the NH2-terminal, the central, and the COOH-terminal regions of the signal sequence to be investigated during insertion into the membrane of the endoplasmic reticulum (ER). We found that different regions of the nascent chains were photocross-linked to different ER proteins. The TRAM protein (translocating chain-associating membrane protein) contacts the NH2-terminal region of the signal sequence while the mammalian Sec61p contacts the hydrophobic core of the signal sequence and regions COOH-terminal of this. These results suggest that the ER translocation complex is composed of heterologous protein subunits which contact distinct regions of nascent polypeptides during their membrane insertion.

The translocation of proteins across the membrane of the rough endoplasmic reticulum (ER), or their insertion into it, is mediated by a series of sequential protein-protein interactions. The first step in this process requires the targeting of the protein to the ER membrane. ER-specific signal sequences present within the nascent chain of proteins (von Heijne, 1988) are recognized by a cytosolic ribonucleoprotein complex, the signal recognition particle (SRP), which functions to target the resulting nascent chain-ribosome-SRP complex to the ER membrane. Specific binding of the nascent chain-ribosome-SRP complex to the ER membrane is mediated by a membrane-bound receptor, the docking protein (or SRP receptor) (Rapoport, 1990). Following interaction with the docking protein, the nascent chain is released from SRP, and membrane insertion is initiated (High and Dobberstein, 1992). Recent electrophysiological data suggest that subsequent translocation across the membrane occurs via a protein-conducting channel (Simon and Blobel, 1991).

Photocross-linking has been successfully used to analyze the interactions of nascent chains during their insertion into the membrane of the ER (High et al., 1991; Krieg et al., 1989; Wiedmann et al., 1987). Two groups of ER proteins have been identified as being adjacent to translocation intermediates of both secretory and membrane proteins (for recent review see High, 1992). Glycoproteins of 35-39 kDa were found to be the major cross-linking partners of short nascent chains of the secretory protein preprolactin (Görlich et al., 1992a; Krieg et al., 1988; Wiedmann et al., 1987). The principal glycoprotein which is cross-linked to these nascent chains is the 36-kDa TRAM protein (Görlich et al., 1992a). Glycoproteins of similar molecular weight have also been found in the proximity of some membrane proteins, although often as minor components (High et al., 1991; Thrift et al., 1991). A 37-kDa nonglycosylated protein (P37) has been shown to be the major cross-linking partner of membrane proteins with uncleaved signal-anchor sequences (High et al., 1991, 1993), and a similar protein was observed when two other membrane proteins were used (Thrift et al., 1991). When longer nascent preprolactin chains are analyzed by photocross-linking an interaction with nonglycosylated proteins is also detected (Krieg et al., 1989).

The major nonglycosylated protein which is cross-linked to these nascent preprolactin chains has recently been identified as the mammalian homologue of Sec61p (Görlich et al., 1992b). When cross-linking analysis with a homobifunctional reagent was performed a 34-kDa nonglycosylated protein (imp34) was the only detectable neighbor of preprolactin. This interaction was independent of the length of nascent chain which was used (Kellaris et al., 1991).

The biosynthetic photocross-linking approach used to date has depended upon the incorporation of modified lysine residues during cell-free translation (Krieg et al., 1986; Kurzchalia et al., 1986). Since several lysine residues are often present in a polypeptide, it is difficult to identify the ER components which contact specific regions of the nascent chain. In addition, acylation of the ε-amino group of lysyl residues removes a positive charge, and this may result in altered interactions. Although in the case of preprolactin it has proved possible to distinguish photocross-linking via the signal sequence from...
photocross-linking via the rest of the nascent chain (Krieg et al., 1989; Wiedmann et al., 1989), it has not been possible to identify components interacting with distinct parts of the signal sequence during membrane insertion.

In this study we make use of a novel method for the incorporation of a photocross-linkable amino acid at a single predetermined position within a nascent chain. A cell-free translation system is supplemented with an amber suppressor tRNA (tRNA^{am}^{m}) chemically charged with the photosensitive amino acid L-4’-[(3-(trifluoromethyl)-3H-diazirin-3-yl)phenylalanine (tTmdPhe). This amino acid is incorporated into the nascent chains in response to a UAG stop codon in the mRNA and, upon photocross-linking, forms a covalent cross-link to adjacent components. This technique was applied here to investigate the interactions between three distinct sites within the signal sequence of preprolactin (PPL) and components of the ER membrane translocation complex. During the membrane insertion of the signal sequence, we find clear evidence that different regions are in contact with different ER proteins. The NH2-terminal region of the signal sequence is in contact with a 36-kDa glycoprotein denoted the “translocating chain-associated membraneous” (TRAM) protein (Görlrich et al., 1992a). The remainder of the signal sequence, together with a region COOH-terminal of the signal sequence cleavage site, was found to contact the recently identified mammalian Sec61p (Görlrich et al., 1992b). We propose that the membrane translocation complex of the ER consists of heterologous protein subunits, and that Sec61p is a core component of this complex.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes and yeast tRNA^{m}^{m} were from Boehringer Mannheim GmbH (Mannheim, Germany). T4 RNA ligase was from Pharmacia LKB GmbH (Freiburg, Germany) and pGEM4 from Promega Corporation (Madison, WI). T7 RNA polymerase was from New England Biolabs GmbH (Schwalbach, Germany) and USB GmbH (Bad Homburg, Germany). Qiaquick columns were from Qiagen GmbH (Düsseldorf, Germany). [35S]Methionine and the site-directed mutagenesis kit were obtained from USB GmbH (Bad Homburg, Germany). RNasin was obtained from Promega Corporation (Madison, WI). T4 RNA ligase was from New England Biolabs GmbH (Schwalbach, Germany) and USB GmbH (Bad Homburg, Germany). ER proteins. The NH2-terminal region of the signal sequence is in contact with a 36-kDa glycoprotein denoted the “translocating chain-associated membraneous” (TRAM) protein (Görlrich et al., 1992a). The remainder of the signal sequence, together with a region COOH-terminal of the signal sequence cleavage site, was found to contact the recently identified mammalian Sec61p (Görlrich et al., 1992b). We propose that the membrane translocation complex of the ER consists of heterologous protein subunits, and that Sec61p is a core component of this complex.

Preparation of (Tmd)Phe-tRNA^{m}^{m}-Oligoribonucleotides, pCpA and CpUpApA, were prepared as described elsewhere. T-Lys-(Tmd)phenylalanine and abbreviated suppressor tRNA missing the 3’-terminal dinucleotide pCpA (tRNA^{m}^{m}(-CA)) were prepared as described previously (Baldini et al., 1988; Bruce and Uhlenbeck, 1982; Noren et al., 1988b). The tRNA^{m}^{m}(-CA) was also prepared in vitro as a run-off transcript from the plasmid pYPhe2 and found to function equally well in suppression of TAG stop codons and subsequent photocross-linking. The scheme for the chemical aminoacylation of tRNA^{m}^{m} with (Tmd)Phe is outlined in Fig. 1a. To a solution of 35 mg (94 µmol) of T-Lys-(Tmd)Phe in 160 µl of dry triethylsilane were added 16 mg (94 µmol) of 1,1’-carbonyldiimidazole. After activation of the acid (15 min at room temperature), 160 A260 units of pCpA dissolved in a mixture of 175 µl of water and 190 µl of acetonitrile were added, and the mixture was allowed to react for 4 h at room temperature. The solution was extracted with ethyl acetate, and the aqueous phase, containing unreacted pCpA and acyl-pCpAs, was subjected to reverse phase HPLC using an Aquapore RP300 column (10 µm, 4 × 250 mm). Elution of the products was effected using a gradient of 0-100% acetonitrile in 50 mM ammonium acetate, pH 4.5, over 50 min at 1 ml/min (Fig. 1b). The material in peaks 3 and 4 was pooled and lyophilized to give a mixture

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solution was immediately extracted with phenol (equilibrated with 0.25 M sodium acetate, pH 4.5), phenolchloroform (1:1), and then chloroform. The aqueous phase was further purified by gel filtration using a Bio-Spin 6 column, equilibrated with 250 mM sodium acetate, pH 4.5), and the modified tRNA was then recovered by precipitation with ethanol.

RNA Transcription, Translation, and Photocross-linking—To prepare tRNA* -tRNA* (t-CA) as a run-off transcript the plasmid PVPh2 was first linearized with FokI (Nolen et al., 1988b). The transcription reaction contained 0.13 μg·μl⁻¹ of linearized DNA, 40 μM Tris-HCl, pH 8.0, 20 μM MgCl₂, 5 μM dithiothreitol, 1 μM spermidine, 2 mM each ATP, CTP, GTP, and UTP, 4 units μl⁻¹ ribonuclease inhibitor, 1 μl reactions were incubated at 37 °C for 2 h. The RNA was purified on a Qiagen 100 column as directed by the manufacturer (Diagen) and then used directly for chemical charging.

The plasmid pGEM4PPL was constructed by subcloning the coding region of pSBFP4 (Siegel and Walter, 1985) into pGEM4 as an EcoRI/HindIII fragment under control of the T7 promoter. Stop codons (TAG) were introduced at codons 11, 18, and 25 of the coding region of PPL to give PPL TAG11, PPL TAG18, and PPL TAG25, respectively. PPL AK4, AK9, and AK6 were made by altering codons 4 and 9 of PPL from encoding lysine to encoding arginine and by replacing the arginine codon at position 46 by a lysine codon. The nascent chain was labeled with an [35S]methionine-directed mutant tRNA in the presence of a (Tmd)Phe-tRNA* should be efficient enough to allow visualization of photocross-linking products between the resulting nascent chains and interacting components. The presence of a (Tmd)Phe residue at position 11, 18, or 25 of the PPL nascent chain did not affect the translocation of the full-length protein product across microsomal membranes or influence the accompanying signal sequence cleavage (data not shown). We concluded that the truncated PPL nascent chains to be used for photocross-linking analysis would represent true intermediates of the translocation pathway used by the wild type protein.

During Membrane Insertion Different ER Proteins Are in Contact with Different Regions of the Nascent Preprolactin—Upon the addition of rough microsomes to a nascent chain—ribosome-SRP complex the SRP is released and the nascent chain contacts membrane proteins. The continued presence of the ribosome retains the nascent chain in the translocation site and allows for ER components close to the membrane-inserting nascent chain to be detected (Krieg et al., 1989; Wiedmann et al., 1987). We added rough microsomes to the various PPL nascent chains and photocross-linked them to the ER components with which they were in contact. Since the nascent chains are all of the same length, they should all be trapped at the same stage of the translocation process. Thus, using the site-specifically introduced photocross-linking probes, the nearest neighbors of successive regions of the nascent chain could be determined. As a control we used the modified lysine mediated photocross-linking method for the PPL AF, which had been described previously (Krieg et al., 1989; Wiedmann et al., 1987). The modified lysine residues from which photocross-linking occurs are present exclusively at the NH₂-terminal side of the hydrophobic region of the signal sequence (see Fig. 6). After photocross-linking of the different PPL nascent chains to ER components, the products were extracted with sodium carbonate solution and centrifuged to separate integral membrane proteins, which are in the resulting pellet, from soluble proteins which remain in the supernatant (Fujiki et al., 1982). The different PPL TAG proteins gave photocross-linking products of different mobilities on SDS-polyacrylamide gels (Fig. 3, lanes 2–4), although the mobilities of the nascent chains were all the same (Fig. 2b, lanes 6–8, and Fig. 3, lanes 2–4). The induction of photocross-linking to adjacent components via the incorporated (Tmd)Phe residue was dependent upon UV irradiation as expected (data not shown). When PPL TAG11 was used for analysis, a 43-kDa photocross-linking product was observed (Fig. 3, lane 2) which was almost identical in size to the product obtained when modified lysine residues were used for photocross-linking (Fig. 3, lane 1). The latter represents the 8-kDa PPL AF covalently attached to a component of about 35 kDa (Krieg et al., 1989; Görlich et al., 1992a). This component consists of at least two glycoproteins, the bulk of the nascent chain being photocross-linked to the translocating chain-associating membrane (TRAM) protein (Görlich et al., 1992a) and a minor portion to the SSRO subunit (Wiedmann et al., 1987).

Signal Sequence Interactions with ER Proteins
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FIG. 2. a, outline of PPL85 polypeptide (top) and PPL85 transcripts used to test stop codon suppression by Phe-tRNA<sup>sup</sup>. Phe-tRNA<sup>sup</sup> was prepared exactly as (Tmd)Phe-tRNA<sup>sup</sup> except that Phe was used in place of (Tmd)Phe. The black box indicates the hydrophobic core of the signal sequence and the site of signal peptidase cleavage is shown (SPase). The codon for amino acids 11, 18, and 25 of normal ppls was replaced in the transcript by a UAG stop codon as indicated (TAG11, TAG18, and TAG25). b, transcripts coding for PPL85 TAG11, PPL85 TAG18, or PPL85 TAG25 were translated in the absence (lanes 1–4) and presence (lanes 5–8) of Phe-tRNA<sup>sup</sup> (tRNA<sup>sup</sup>). The resulting translation products were analyzed on a 22% polyacrylamide gel containing 6 M urea and subjected to fluorography. The calculated number of amino acids in the resulting polypeptides is indicated on the right.

TAG18 was used the major photocross-linking product had an estimated molecular mass of 45 kDa (Fig. 3, lane 3), suggesting the 8-kDa nascent chain was covalently attached to a component of 37 kDa. The photocross-linking pattern observed with PPL TAG25 showed a major photocross-linked product of 45 kDa together with a weaker 43-kDa product (Fig. 3, lane 4). This suggested that the bulk of the nascent chain was cross-linked to a 37-kDa protein, whereas a fraction was cross-linked to a 35-kDa protein.

Identification of the ER Proteins Which Contact Nascent PPL—The 35-kDa ER proteins, which are the major photocross-linking partners of PPL AF, are known to be glycoproteins, since they bind to immobilized lectins (Gorlich et al., 1992a; Krieg et al., 1989; Wiedmann et al., 1987). Neither the PPL AF TAG18 nor the PPL AF TAG11, or PPL AF TAG25 photocross-linked components were found to bind immobilized lectins, indicating that the photocross-linked components are not glycoproteins (data not shown). To further characterize the protein components which are photocross-linked to the different regions of the PPL nascent chain, the products were immunoprecipitated using antibodies specific for the TRAM protein, the major ER protein found photocross-linked to short truncations of the PPL nascent chain (Gorlich et al., 1992a). The photocross-linking products between PPL AF and PPL AF TAG11 and the 35-kDa ER component were efficiently immunoprecipitated by antibodies against the TRAM protein, the major ER protein found photocross-linked to short truncations of the PPL nascent chain (Gorlich et al., 1992a). The photocross-linking products between PPL AF and PPL AF TAG11 and the 35-kDa ER component were efficiently immunoprecipitated by antibodies against the TRAM protein (Fig. 4, lanes 3 and 6). The slight difference in mobility of the PPL AF-TRAM protein cross-link and the PPL AF TAG11-TRAM protein cross-link may be due to the nascent chains being photocross-linked to different regions of the TRAM protein. In contrast, the PPL AF TAG18, PPL AF TAG25, and PPL AF ΔK4 ΔK9 K46 photocross-linked components showed no reactivity with the anti-TRAM antibodies (Fig. 4, lanes 9, 12, and 15).

The 37-kDa protein which was photocross-linked to PPL AF TAG18, PPL AF TAG25, and PPL AF ΔK4 ΔK9 K46 was similar in apparent size, and lack of glycosylation, to the Saccharomyces cerevisiae Sec61p (Müsch et al., 1992; Sanders et al., 1992) and its recently identified mammalian homologue...
(Görlich et al., 1992b). We therefore repeated the analysis of all the photocross-linking products using antibodies specific for mammalian Sec61p (Görlich et al., 1992b). The major 45-kDa photocross-linking products obtained with PPL AF TAG18, PPL AF TAG25, and PPL AF ∆K4 ∆K9 K46 were all efficiently immunoprecipitated with these anti-Sec61p antibodies (Fig. 5, lanes 9, 12, and 15). Thus the 37-kDa nonglycosylated ER protein photocross-linked to these nascent chains was identified as Sec61p. A small amount of the 45-kDa photocross-linking product was also immunoprecipitated when PPL AF and PPL AF TAG11 were used for photocross-linking (Fig. 5, lanes 3 and 6). This is consistent with the presence of a faint 45-kDa photocross-linking product which is observed with these nascent chains (Fig. 4, lanes 1 and 4; Görlich et al., 1992b) and which is not immunoprecipitated by the anti-TRAM antibodies (Fig. 4, lanes 3 and 6).

We conclude that two different ER proteins are in contact with different regions of the translocation intermediate of the nascent secretory protein PPL. One of these is the TRAM protein (Görlich et al., 1992a), whereas the other is mammalian Sec61p (Görlich et al., 1992b) (see Fig. 6).

**DISCUSSION**

**Site-specific Photocross-linking**—We have demonstrated here the site-specific incorporation of the photoactivatable amino acid (Tmd)Phe into PPL nascent chains and the successful photocross-linking of these nascent chains to components of the ER translocation complex. The approach used is entirely different from that developed previously (Krieg et al., 1986; Kurzchalia et al., 1986) and offers several advantages and the potential for further developments.

A key element of the present study was the preparation of amber suppressor tRNA with (Tmd)Phe. This was accomplished by using the general scheme of Hecht and colleagues (Heckler et al., 1984; Payne et al., 1987; Pezuto and Hecht, 1980), which involved T4-RNA ligase-mediated coupling of 3′(2′)-O-aminocetyl-pCpA to a tRNA lacking the 3′-terminal cytosine and adenosine moieties. The procedure used for the preparation of 3′(2′)-O-(Tmd)phenylalanyl-pCpA is essentially that described by Baldini et al. (1988) except that the acylation reaction was carried out in a different solvent system (tetrahydrofuran/acetonitril/water). This results in increased yields of the desired 3′(2′)-O-(Tmd)phenylalanyl-pCpA. A highly efficient route for the chemical aminoacylation of tRNAs has recently been reported by Robertson et al. (1991). Unfortunately the protection/deprotection scheme which
problem when antibodies recognizing the cross-linked com-
phenylalanine, designed for the chemical
easily detect cross-linking products.

Escherichia coli extracts and reticulocyte lysate (Bain et al.,
1989, 1991a, 1992; Chung et al., 1993; Ellman et al., 1991,
1992a; Ellman et al., 1992b; Mendel et al., 1992; Noren et al.,
1989a, Robertson et al., 1991). In this study we have used a
wheat germ cell-free translation system, supplemented with
chemically charged suppressor tRNA prepared either by the
anticodon loop replacement procedure (Bruce and Uhlenbeck,
1982; Noren et al., 1989a) or by run-off transcription (Noren
et al., 1989b, Bain et al., 1991b). Our results demonstrate that
enough protein could be generated with the present system to
easily detect cross-linking products.

(Tmd)Phe is a carbene-yielding structural analogue of
phenylalanine, designed for the chemical or biosynthetic in-
corporation into peptide and protein photoaffinity reagents (Nassal,
1984). Because of its reasonably small size, (Tmd)Phe may be incorporated into proteins without seri-
ously affecting either the structure of the protein or its inter-
action with adjacent components. Upon photolysis, a very
reactive singlet carbene is generated which is capable of inserting even into aliphatic CH bonds (Brunner et al.,
1980; Nassal, 1984). The short half-life of the carbene (estimated
to be less than 10^-9 s) ensures that it cross-links only to
components in direct contact. Cross-linking as a result of
random collisional encounters is very unlikely to occur.

One of the limitations of current biosynthetic photocross-
linking approaches is the lack of efficient methods to identify and
analyze cross-linked components. This is a particular
problem when antibodies recognizing the cross-linked com-
ponent are not available or when the precise sites of cross-
linking need to be determined. To this end we are developing
methods which allow the site-specific incorporation of a cleav-
able, photoactivatable, and radioactively labeled amino acid of high specific radioactivity. The ultimate goal is to combine
the present technology with developments in conventional
cross-linking (label transfer cross-linking). First promising
steps in this direction have been made with the preparation
of cysteinyl-tRNA<sup>smp</sup>. Alkylation of the thiol group should
provide a simple and general method to introduce the required multifunctional residue. While site-directed incorporation of photoactivatable amino acids is experimentally more demanding than previous approaches, it has a very
much wider potential for studying biological questions relat-
ing to protein-protein interactions.

**Membrane Components in Contact with Nascent PPL**—The immediate environment of a nascent secretory protein as it is
translocated across the ER membrane has been investigated in several ways. One approach has been to generate translo-
cation intermediates of different lengths and then analyze
their next neighbors by photocross-linking. A detailed study of this kind by Krieg et al. (1989) showed that the glycoprotein
mp39 was in proximity to nascent chains of PPL ranging from 86 to 131 amino acids in length. As the length of the nascent chain was increased, the number of cross-linked proteins increased, and nonglycosylated proteins were ob-
served in addition to the glycoproteins. The interpretation of
the results was complicated by the fact that when longer chain
lengths were used, the number of lysine residues from which
photocross-linking to ER components could occur was
increased.

We have kept the length of the nascent chain in the translocation intermediate of PPL constant (PPL AF = 70
amino acids) and moved the position of the photocross-linking reagent within the nascent chain (Fig. 6). Our results show
that the type of ER protein which is photocross-linked to the
nascent chain is dependent upon the region of the nascent chain from which the photocross-linking occurs. The simplest
interpretation is that the different regions of the nascent chain are in proximity to different ER proteins. However, it
is also possible that the three modified amino acids placed into the signal sequence are facing in different lateral direc-
tions and that different sides of the nascent chain contact different proteins. If more than one population of membrane-
inserted signal sequences exists, then they could each contact one of the two different ER proteins via different regions of the signal sequence and also generate the observed results. Further studies will be necessary to formally disprove any of
these possibilities.

At least two different proteins were found to be in contact with different regions of the nascent chain. The NH₂ terminus of PPL (PPL AF and PPL AF TAG11) was photocross-linked to the TRAM protein (Görlich et al., 1992a). A small propor-
tion of nascent chain is also likely to be cross-linked to the previously identified 35-kDa glycoprotein SSSRs (Görlich et al.,
1992a; Wiedmann et al., 1987). The mp39 glycoprotein
which is photocross-linked to the nascent chain of PPL (Krieg et al., 1989) probably represents both of these components.

When the photocross-linking group was placed at the center of the hydrophobic part of the PPL signal sequence (PPL AF
TAG18), only the product of cross-linking between the nas-
cent chain and mammalian Sec61p could be detected. Pho-
tocross-linking from a position COOH-terminal of the hydro-
phobic part of the signal sequence (PPL AF TAG25) and
from a region after the signal peptidase cleavage site (PPL
AF AK4 AK9 K46) also showed Sec61p as the major cross-
linking partner.

In addition to the identification of mammalian Sec61p by
Görlich et al. (1992b), at least two other studies have found nonglycosylated proteins as a major cross-linking partner of
PPL nascent chains during their membrane insertion (Kel-
laris et al., 1991; Krieg et al., 1989). Although further studies are required, it seems likely that these components also rep-
resent cross-linking to the mammalian Sec61p.
Several lines of evidence suggest that the complex which mediates the ER translocation of secretory proteins is either the same as that which is responsible for the insertion of membrane proteins or that it utilizes some common components. We find that Sec61p is in contact with the secreted protein PPL at various stages during its translocation across the membrane (this work; Görlich et al., 1992b) and also with several membrane proteins during their insertion into the ER (High et al., 1991b; High et al., 1993). In S. cerevisiae mutations in the SEC61, SEC62, and SEC63 genes cause defects in both the translocation of secreted proteins (Deshaies and Schekman, 1989; Sadler et al., 1989; Rothblatt et al., 1989) and the insertion of membrane proteins (Green et al., 1992; Stirling et al., 1992), consistent with a single ER complex mediating both protein translocation and membrane protein insertion.

Our results suggest that the ER translocation complex consists of heterologous protein subunits and that Sec61p and TRAM are two components of this complex. Indeed the TRAM protein has already been shown to be required for the in vitro translocation of some secreted proteins (Görlich et al., 1992a). In S. cerevisiae translocation intermediates of a secretary protein can be cross-linked to Sec61p (Müsch et al., 1992; Sanders et al., 1992). Sec61p had been identified previously as a gene product essential for the efficient translocation of secretory proteins into the ER (Deshaies and Schekman, 1987). The frequency with which interactions between nascent secretory or membrane proteins and Sec61p are observed support the view that this protein plays a key role in membrane translocation and membrane insertion. We propose that Sec61p is a core component of the mammalian ER translocation machinery and as such be absolutely required for the process to occur (High, 1992). This is consistent with the proposal that Sec61p alone, or in combination with other unidentified ER proteins, mediates the translocation of proteins across the ER membrane (see Rapoport, 1992). There is sufficient sequence similarity between mammalian Sec61p and bacterial SecY to suggest that the mechanism of protein translocation may have been conserved (see Görlich et al., 1992a). In S. cerevisiae Sec61p is associated with Sec62p, Sec63p, and two uncharacterized proteins (Deshaies et al., 1991). It is not presently known whether mammalian homologues of these S. cerevisiae proteins exist.

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