Mammalian Sec61 Is Associated with Sec62 and Sec63*

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In yeast, efficient protein transport across the endoplasmic reticulum (ER) membrane may occur co-translationally or post-translationally. The latter process is mediated by a membrane protein complex that consists of the Sec61p complex and the Sec62p-Sec63p subcomplex. In contrast, in mammalian cells protein translocation is almost exclusively co-translational. This transport depends on the Sec61 complex, which is homologous to the yeast Sec61p complex and has been identified in mammals as a ribosome-bound pore-forming membrane protein complex. We report here the existence of ribosome-free mammalian Sec61 complexes that associate with two ubiquitous proteins of the ER membrane. According to primary sequence analysis both proteins display homology to the yeast proteins Sec62p and Sec63p and are therefore named Sec62 and Sec63, respectively. The probable function of the mammalian Sec61-Sec62-Sec63 complex is discussed with respect to its abundance in ER membranes, which, in contrast to yeast ER membranes, apparently lack efficient post-translational translocation activity.

The mammalian Sec61 complex consisting of Sec61α, Sec61β, and Sec61γ has been identified as a crucial membrane component involved in the signal recognition particle (SRP)-dependent co-translational protein translocation across the endoplasmic reticulum (ER) membrane (for review see Ref. 2). The Sec61 complex forms the hydrophilic pore in the membrane through which the nascent polypeptide is translocated (3–6), and it is responsible for the tight binding of the ribosome to the ER membrane during the co-translational transport process (7). Moreover, the Sec61 complex is involved in the recognition of the signal sequence regulating the insertion of the nascent polypeptide chain into the translocation channel (8, 9). The ribosome-bound Sec61 complex is in spatial proximity to several other membrane components that interact with the nascent polypeptide chain during its co-translational translocation. These components include the translocating chain-associated membrane protein (TRAM) (10), the signal peptidase complex (SPC) (11–13), the oligosaccharyltransferase complex (14), the translocon-associated protein (TRAP) complex (15), and the ribosome-associated membrane protein 4 (RAMP4) (4, 16). Whereas the functions of the SRP and the oligosaccharyltransferase complex are well established, the role of the other components is at best poorly understood or completely unknown. Vectorial co-translational protein translocation into the ER can be reconstituted in the absence of chaperones using proteoliposomes consisting exclusively of the SRP receptor (essential for the SRP-dependent targeting step (17)), the Sec61 complex, and TRAM (4). However, other data suggest that chaperones in the ER lumen play a stimulatory role during translocation in vitro (18, 19). Moreover, the Hsp70 homolog BiP is likely involved in the formation of a tight seal that blocks ion transport across the Sec61 complex in the absence of protein translocation (20).

In the yeast Saccharomyces cerevisiae, in the absence of tightly bound ribosomes, the trimeric Sec61p complex is found associated with other polypeptides (Sec62p, Sec63p, Sec71p, and Sec72p) (21, 22), which together form the Sec complex (23). The yeast Sec complex is essential and sufficient for the post-translational protein translocation into the ER (24). Sec63p has a DnaJ-like domain located in the ER lumen (25), which recruits the Hsp70 homolog Kar2p to the translocation sites. The DnaJ domain of Sec63p and Kar2p form a molecular ratchet that is responsible for the ATP-dependent vectorial movement of the polypeptide into the ER lumen (26). In contrast to Sec62p, Sec63p, and Kar2p, neither Sec71p nor Sec72p are essential for post-translational translocation (27–29). It is possible that components of the Sec complex are also involved in other cellular processes. Mutations in Kar2p, Sec71p, Sec72p, and Sec63p affect karyogamy in yeast (30, 31), and there is genetic evidence that Sec61p, Sec63p, and Kar2p are involved in the ubiquitin- and proteasome-dependent degradation of proteins at the ER (32–34).

In mammalian cells it is also the case that not all Sec61 complexes are tightly associated with ribosomes (35). To date, however, homologs of neither the yeast Sec62-Sec63 complex nor other membrane components found preferentially associated with ribosome-free Sec61 complexes have been identified. We therefore set out to identify and characterize such proteins of the mammalian ER.

We show here, that the mammalian ER contains proteins that display structural homology to the yeast Sec62p and Sec63p. Both proteins are expressed ubiquitously, and their abundance is similar to that of known components of the ER translocation machinery. To gain an indication as to the function of these proteins, we analyzed their molecular environment in the ER using different biochemical methods. We found

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‡ The abbreviations used are: SRP, signal recognition particle; BiP, immunoglobulin-binding protein; eq, equivalent of membranes (1); ER, endoplasmic reticulum; PK-RM, puromycin/high salt-treated rough membranes; RAMP, ribosome-associated membrane protein; RM, rough membranes; SPC, signal peptidase complex; TRAM, translocating chain-associated membrane protein; TRAP, translocon-associated protein; PAGE, polyacrylamide gel electrophoresis; deoxy-BIGGHP, N,N-bis-(3-n-glucosaminidopropyl)deoxycholamide.

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that Sec62 and Sec63 are associated with Sec61 complexes, indicating that these proteins might be involved in transport processes similar to those performed by the yeast Sec complex. The association was only detectable in the absence of tightly bound ribosomes. This suggests that the function of Sec62 and Sec63 is most likely not directly linked to the co-translational protein translocation across the mammalian ER.

**Experimental Procedures**

**cDNA Cloning—**Human Sec63 cDNA was recovered by polymerase chain reaction using the oligonucleotides GTCCACCAATATGCCGACTGCATC and ATGGCCGGGACGACGTTCCAG and HeLa cDNA as template. Design of the oligonucleotide sequences was based on information from two human expressed sequence tags, GenBankTM accession numbers N79940 and A227342, respectively. Two of the obtained clones were sequenced on both strands.

Partial clones of human Sec62 were isolated by screening a HeLa cDNA library (Stratagene) with the oligonucleotide GTCCACCAATATGCCGACTGCATC from a human expressed sequence tag (GenBankTM accession number H17484) using standard protocols. The missing 5′ ends were obtained by rapid amplification of cDNA ends using a fetal brain Marathon-cDNA (CLONTECH). Clones covering the entire open reading frame were subsequently obtained by polymerase chain reaction using the same cDNA, and two of them were sequenced.

Sequence analyses were performed using software from PCGENE and from the web page of the National Center for Biotechnology Information. The sequences of human Sec62 and Sec63 were deposited in the GenBankTM data base under the accession numbers U93239 and AF100141, respectively.

**Antibodies—**The following peptide-specific polyclonal antibodies were used: anti-Sec61b raised against the amino terminus of Sec61b (35), anti-Sec61a raised against the carboxyl terminus of Sec61a (4), anti-SRP-receptor α raised against the position 137–150 of the protein, anti-TRAP raised against the carboxyl terminus of TRAM (10), anti-TRAPb raised against the carboxyl terminus of TRAPb (36), anti-Sec62 raised against the carboxyl terminus of human Sec62 (C7PKSSHEKKS), and anti-Sec63 raised against the position 504–523 of human Sec63 (CTNKRSTRGGSKQSRKPRK). Monoclonal antibodies against the rat NADPH P-450 were purchased from Dai-ichi Pure Chemical Co., Ltd.

**Membrane Purification—**To obtain “crude H” membrane fractions, tissues were homogenized in 50 mM HEPES-KOH (pH 7.8), 1 mM EDTA, 320 mM sucrose, and protease inhibitor mix. After centrifugation for 10 min at 2,500 rpm (Sigma 3K12 centrifuge, 4 °C), the supernatant was centrifuged at 100,000 rpm for 1 h (Ti-60 rotor, 4 °C). The resulting pellet was resuspended in the homogenization buffer. Purification of rough membranes (RM) was performed (1). Purification of smooth membranes (SM) was performed (37). 10 g of bovine liver were homogenized in buffer H (50 mM HEPES-KOH (pH 7.8), 150 mM potassium acetate, 10 mM magnesium acetate, 0.75 mM salt (PK-RM) and solubilized with digitonin. The detergent mixture was incubated for 1 h at 4 °C and 70,000 rpm, Ti70 rotor), the supernatant was applied to a 25–50% (w/v) sucrose gradient containing buffer H with 1.5 M sucrose and 2.0 M sucrose. The gradient containing buffer was formed by 250 mM potassium acetate, 10 mM magnesium acetate, 10% (w/v) glycerin, 5 mM β-mercaptoethanol, and protease inhibitor mix to a final concentration of 0.75 eq/µl. After a centrifugation step (1.5 h at 4 °C and 70,000 rpm, Ti70 rotor), the supernatant was applied to a HiTrap Q column (Amersham Pharmacia Biotech). The column was washed with buffer W (0.5% digitonin, 50 mM HEPES-KOH (pH 7.8), 10% (w/v) glycerin, 5 mM β-mercaptoethanol, and protease inhibitor mix) supplemented with 500 mM potassium acetate. The elution was performed with increasing concentrations of salt in step fractions from 0.6 to 1.2 M potassium acetate in buffer W. The eluate was then divided with 2 volumes of buffer W containing protease inhibitor mix and passed over an anti-Sec61b antibody column. The bound material was eluted with 280 mM potassium acetate and protease inhibitor mix.

**Chemical Cross-linking—**Canine RM in 50 mM HEPES-KOH (pH 7.8), 150 mM potassium acetate, 5 mM magnesium acetate, 200 mM sucrose, and protease inhibitor mix were treated with 50 µM bismaleimidohexane (Pierce) (stock solution: 1 mM bismaleimidohexane in dimethylformamide) for 25 min at 0 °C. The reaction was quenched by addition of 280 mM β-mercaptoethanol.

**Miscellaneous—**Immuno blotting and immunoprecipitation were carried out as described (10). Partial protein sequences were obtained from purified proteins as described (24). The final concentration of protease inhibitor mix was 10 µg/ml leupeptin, 5 µg/ml chymostatin, 2 µg/ml antipain, and 10 µg/ml aprotinin. Protein concentrations were determined by quantitative immunoblotting using ECL peroxidase (NEN Life Science Products) and a CSC chemiluminescence camera (Rayment). Dighton was purchased from Sigma, deoxy-BIGCHAP from Calbiochem, and saponin from Roth.

**Results**

**Identification and Cloning of Human Homologs of Sec63p and Sec62p—**In order to identify proteins that are associated to the Sec63 complexes in the absence of ribosomes, bovine rough microsomes were treated with puromycin in the presence of 500 mM salt (PK-RM) and solubilized with digitonin. The detergent extract was bound to anti-Sec61b antibodies (Fig. 1). The affinity-purified fraction contained the components of the trim eric Sec63 complex as well as two proteins approximately 80 and 97 kDa in size (Fig. 1, lane 4). Peptides derived from both proteins were subjected to Edman degradation. The 80-kDa peptide was identified as BiP. The peptide sequence of the 97-kDa protein (Fig. 2A) corresponded to a group of human expressed sequence tags in the GenBankTM database. An analysis of these sequences revealed that they belonged to a cDNA that has some homology to yeast Sec63p (about 20% identical amino acids). The entire coding region of this cDNA was cloned and sequenced. The deduced protein sequence of...
centrifugation. The supernatant (extract) was applied to an anti-Sec61 antibody column. After washing (wash), the bound material was eluted with the peptide against which the antibodies were raised (eluate). Samples corresponding to 20 eq (extract and flow-through) or 450 eq (wash and eluate) of the starting material were separated by SDS-PAGE and stained with Coomassie Blue. Protein bands were analyzed by Edman degradation of fragments obtained after digestion with trypsin. The peptides obtained from BiP were TKPYIQVDVG and AVEEKI; peptides obtained from Sec63 are indicated in Fig. 2.

Fig. 1. Identification of mammalian Sec63. Bovine PK-RM were solubilized with digitonin, and non-solubilized material was removed by centrifugation. The supernatant (extract) was applied to an anti-Sec61 antibody column. After washing (wash), the bound material was eluted with the peptide against which the antibodies were raised (eluate). Samples corresponding to 20 eq (extract and flow-through) or 450 eq (wash and eluate) of the starting material were separated by SDS-PAGE and stained with Coomassie Blue. Protein bands were analyzed by Edman degradation of fragments obtained after digestion with trypsin. The peptides obtained from BiP were TKPYIQVDVG and AVEEKI; peptides obtained from Sec63 are indicated in Fig. 2.

carboxyl terminus of 200-kDa proteins of U5 small nuclear ribonucleoprotein.

human Sec63 contains all peptides obtained from the 97-kDa protein (Fig. 2A). Similar to the yeast Sec63p, the human Sec63 and the homologs from Arabidopsis thaliana and Caenorhabditis elegans have a DnaJ domain and three membrane-spanning domains (Fig. 2A). However, several residues proven to be critical for the interaction of the DnaJ domain of yeast Sec63p with Kar2p are not strictly conserved (38). At the primary structure level the most conserved part is predicted to be located in the ER lumen, spanning the DnaJ domain through to the end of the third proposed membrane anchor (about 40% identity between yeast and human). Remarkably, the carboxyl-terminal 500 amino acids of Sec63 proteins from higher eukaryotes form a sequence motif that is found twice in the carboxyl terminus of 200-kDa proteins of U5 small nuclear ribonucleoprotein.

We postulated that mammals may also contain homologs of the other proteins present in the yeast Sec62p-Sec63p subcomplex. Therefore we screened the GenBank™ data base and identified several partial human cDNA sequences that displayed homology to Sec62p. Based on this information we cloned and sequenced cDNAs that contained the entire open reading frame of the gene. While this work was in progress, a complete human Sec62 cDNA was published (39). Fig. 2B shows the alignment of the human protein with homologous proteins from the invertebrate Drosophila melanogaster and C. elegans and from the yeast species Schizosaccharomyces pombe, Yarrowia lipolytica, and S. cerevisiae. In all cases, Sec62 is predicted to have two membrane-spanning segments. The domains flanking the membrane anchors, including the intervening luminal domain, display a high degree of conservation in their primary structure among all proteins analyzed (34% identity between yeast and human Sec62). Regions that are closer to the termini of Sec62, show a striking similarity exclusively to the homologous animal proteins. Based on the sequence information peptides were designed to raise antibodies against Sec62 and Sec63 (Fig. 2).

Sec62 and Sec63 Are Not Associated with Membrane-bound Ribosomes—In order to gain an indication of the possible function of the two proteins, we next analyzed their molecular environment. First we tested whether or not Sec62 and Sec63 are associated with membrane-bound ribosomes characteristic of Sec61α, TRAPα and other ribosome-associated membrane proteins (RAMPs) (4). Rough microsomes were solubilized with digitonin in a buffer containing 450 mM potassium acetate and separated by sucrose gradient centrifugation (Fig. 3). Most of Sec61α and Sec61β and nearly 50% of TRAPα were found in fractions 1–10 co-migrating with the ribosomes as has been reported previously (35). In contrast, Sec62, Sec63, TRAM, and the 25-kDa subunit of the SPC remained in the ribosome-free fractions 11–19. Similar results were obtained if membranes were solubilized with the detergent deoxy-BIGCHAP (not shown and Fig. 5B, lane 3), with the exception that TRAPα was predominantly found in the ribosome-free fractions (not shown).

Sec62 and Sec63 Associates with Ribosome-free Sec61 Complexes—We subsequently chose to investigate the molecular environment of Sec62 and Sec63 in the membrane. The purification of mammalian Sec63 by an anti-Sec61b antibody column indicated that the two proteins are in a complex (Fig. 1). To confirm this result and to identify further proteins of the ER membrane that are associated with mammalian Sec62 or Sec63, we performed immunoprecipitation experiments using anti-Sec62 and anti-Sec63 antibodies. Bovine RM were first treated with saponin in the presence of 0.8 M salt to obtain membranes enriched in integral membrane proteins. These membranes were solubilized with digitonin, and the extract was bound to the antibody column (Fig. 4A). The proteins that eluted from the anti-Sec63 antibody column were analyzed by peptide sequencing (Fig. 4A, lane 1). Sec63, Sec61α, Sec61β, Sec61γ, and a contamination with immunoglobulins were detectable, thus confirming the association between Sec63 and the Sec61 complex. No other proteins were present in significant amounts in the eluate. Material that eluted from the anti-Sec62 antibody column contained exclusively the Sec62 protein (Fig. 4A, lane 2). To find proteins that interacted with Sec62, we repeated the immunoprecipitation experiments using the detergent deoxy-BIGCHAP. RM were solubilized; the RAMPs were separated by centrifugation, and the ribosome-free supernatant was applied to the antibody columns. Under these conditions, the anti-Sec61b antibodies did not only precipitate Sec61α and Sec63 but also Sec62 (Fig. 4B, lane 5). Binding of this membrane extract to an anti-Sec63 antibody column also precipitated about 10% of Sec62, in addition to Sec61α and Sec61β (Fig. 4B, lanes 7 and 8). In both cases other proteins of the translocation site such as TRAM or SRP receptor α were not co-precipitated.

Together these data indicate that both Sec62 and Sec63 form a complex with Sec61 complexes. In agreement with the results of the sucrose gradient centrifugation (Fig. 3), these complexes could be purified from ribosome-free supernatants of RM (Fig. 4). A caveat in these experiments was that the amount of Sec62 precipitated was very sensitive to the salt concentration used for the solubilization of the membranes (not shown). To confirm the association of Sec62 with the Sec61 complex, we therefore performed cross-linking experiments using canine RM and the chemical cross-linker bismaleimidoxyane. We observed several cross-linked products between Sec62 and other proteins in immunoblots (Fig. 5, lane 4). Peptide sequencing of the main
FIG. 2. Protein sequences of human Sec62 and Sec63. A, alignment of Sec63 with homologous sequences. B, alignment of Sec62 with homologous sequences. Identical amino acid residues are indicated by asterisks, and similar amino acid residues are indicated by colons. Putative membrane spanning segments are written black on gray. The region that displays the highest conservation among all proteins is underlined. The DnaJ domain of Sec63 proteins is framed in black. Partial peptide sequences obtained by Edman degradation of the purified proteins are indicated by E, and peptides used to raise antibodies are indicated by #. A, HsSec63, Homo sapiens Sec63 (GenBank™ accession number AF100141); CeSec63, C. elegans (DDBJ/GenBank™/EBI Data Bank accession number AL032652); AtSec63, A. thaliana (GenBank™ accession number AAD55642); ScSec63, S. cerevisiae Sec63p (DDBJ/GenBank™/EBI Data Bank accession number X16388). B, HsSec62, H. sapiens Sec62 (GenBank™ accession number U93239); DmSec62, D. melanogaster Dtrp1 (GenBank™ accession number AC005464); CeSec62, C. elegans (DDBJ/GenBank™/EBI Data Bank accession number Z70034); SpSec62, S. pombe (DDBJ/GenBank™/EBI Data Bank accession number Z91962); YlSec62, Y. lipolytica Sec62 (DDBJ/GenBank™/EBI Data Bank accession number X99537); ScSec62, S. cerevisiae Sec62p (DDBJ/GenBank™/EBI Data Bank accession number X16666).

FIG. 3. Sec62 and Sec63 are not ribosome-associated membrane proteins. RM solubilized in high salt digitonin buffer were separated by sucrose gradient centrifugation. Fractions were analyzed by SDS-PAGE and immunoblotting using antibodies as indicated. Fractions containing ribosomal proteins are indicated by a black frame. P, pellet.
product, which contained about 30% of the Sec62 present in the membranes, identified the β-subunit of the Sec61 complex as the cross-linked partner. To test whether or not the Sec61β cross-linked to Sec62 belongs to a ribosome-bound Sec61 complex, we separated the membrane proteins by centrifugation after solubilization with digitonin into a ribosome-free supernatant and a pellet fraction containing the ribosomes and the RAMPs. The cross-linked product between Sec62 and Sec61β remained in the supernatant (Fig. 5, lanes 5 and 6). An analysis of the same samples by immunoblotting using anti-Sec61β antibodies revealed that the other Sec61β-containing cross-linked products were found in the pellet fraction (Fig. 5, lane 9), suggesting that they were ribosome-associated. Among them was a cross-linked product between Sec61β and SPC25, a protein that in the absence of cross-linker does not behave like a RAMP (see Fig. 3 (13)). This demonstrates that cross-linking of a non-RAMP to a ribosome-associated Sec61β can identify a protein such as a RAMP. Only one band, which according to its mobility in the SDS-PAGE corresponds to the Sec62-Sec61β cross-linking product, was entirely found in the supernatant, indicating that it is not ribosome-bound.

**Further Characterization of the Sec63-Sec61 Subcomplex**—To analyze the Sec61-Sec63 complex in more detail an alternative purification protocol was developed. PK-RM were solubilized with digitonin. The detergent extract was passed over a HiTrap Q column at 0.5 M salt, and the bound material was eluted stepwise with increasing salt concentrations. As expected (4), the bulk of the Sec61 complex did not bind and was therefore found in the flow-through (Fig. 6A, lane 2). However, about 5% of the Sec61 complex eluted at 1.0 M salt together with the bulk of the Sec63 (Fig. 6, A, lane 6, and B, lane 1). This fraction was passed over an anti-Sec61β antibody column. About 30% of the Sec63 was found to bind to the Sec61 complex (Fig. 4C, lane 3). Vice versa, all of the Sec61β in this fraction bound to an anti-Sec63 column (not shown). A Coomassie Blue staining of the recovered Sec61-Sec63 complex after its separation by SDS-PAGE revealed that the preparation did not contain significant amounts of other proteins (Fig. 6B, lane 3). Sec62 remained either in the flow-through or eluted at 0.6 M salt (Fig. 6A, lanes 2 and 4). Immunoprecipitation of these fractions using anti-Sec62 antibodies did not detect proteins associated to Sec62 (not shown). The amount of Sec63 and of Sec61α in four independently purified complex preparations was determined by semi-quantitative immunoblotting (not shown). The molar ratio between Sec63 and Sec61α was in the range between 1.2 and 1 and 1.9 and 1.

**Sec62 and Sec63 Are Ubiquitously Expressed in the Endoplasmic Reticulum**—Finally we wanted to explore the expression pattern of Sec62 and Sec63. First, we performed immunoblot experiments using crude membrane fractions from different rat tissues and rough microsomes derived from different bovine tissues (Fig. 7A). Both proteins were identified in all tissues examined with the highest abundance in samples that also have a high level of Sec61β. Next, we performed a cell fractionation using bovine liver as starting material (Fig. 7B). Nearly all Sec62 and Sec63 was found in the post-mitochondrial supernatant. A separation of this fraction into RM and smooth membranes revealed that both Sec62 and Sec63 were present in the rough ER. Both proteins were also found in the membrane fraction that contained the smooth ER. This fraction, which was essentially free of RM, contained more than 50% of the Sec62. The relative amount of Sec63 in the smooth membranes was significantly lower. Similar results were obtained in fractionation experiments using mouse liver (not shown). The pattern of the intracellular distribution of Sec62 and Sec63 in HepG2 cells observed by immunofluorescence was indistinguishable from that obtained with antibodies against proteins of the ER lumen (not shown). We concluded that most of Sec62 and Sec63 found in the smooth membranes was actually located to the smooth ER.

To estimate the amount of Sec62 and Sec63 in the bovine rough ER, we performed quantitative immunoblotting using purified Sec61 complex, Sec62, and Sec63 as standards (not shown). One equivalent (1) RM contains 0.35–0.65 pmol of Sec62, 0.25–0.5 pmol of Sec63, and 1.1–1.6 pmol of Sec61α.

**DISCUSSION**

The data presented here demonstrate that the mammalian Sec61α can be found in a protein complex with structural similarity to the yeast Sec complex. In addition to the components of the trimeric Sec61 complex, Sec61α, Sec61β, and Sec61γ, this larger complex contains at least two other membrane proteins. These proteins, Sec62 and Sec63, display homology to the yeast proteins Sec62p and Sec63p, respectively.
Remarkably, for both proteins, regions with significant homology were those exposed to the ER lumen or located close to the cytosolic surface of the membrane. The more distal cytosolic parts were very divergent. While this manuscript was in preparation, Skowronek et al. (40) also published the existence of the mammalian Sec63 and showed that it has the same membrane topology as the yeast protein. Both Sec62 and Sec63 were ubiquitously expressed in the rough ER of mammals, and the expression level of Sec62 in a particular tissue is roughly the same in all species tested. However, we cannot exclude that the abundance of Sec63 differs between species, because the epitope recognized by our anti-Sec63 antibodies is not conserved among mammals. Sec62 was also very abundant in smooth membranes that are essentially free of Sec61 complex. This is in agreement with our observation that Sec62 is expressed at high levels in the adrenal gland2 and that the mRNA is abundant not only in liver and pancreas but also in muscle tissues (39).

Although Sec62 and Sec63 were abundantly expressed, the actual concentration of Sec61-Sec62-Sec63 complexes in the ER appears to be relatively low. Regardless of the detergent used, and whether the purification started with RM or with PK-RM, only about 5% of Sec61 and about 30% of Sec63 were found in a complex. In each case the molar ratio between Sec61 and Sec63 in the complex appeared to be 1:1 to 1:2. The binding of Sec63 to the complex was stable, whereas the association of Sec62 with this complex was much weaker. Under optimized purification conditions, we found about 15% of the Sec62 copurified with Sec61. However, in the cross-linking experiment 30% of the Sec62 was linked to Sec61. In the same samples less than 5% of the Sec61 was in proximity to Sec62, similar to that found with Sec63. Therefore the amount of Sec62 and Sec63 in these complexes was likely to be the same. If one assumes that 3 to 4 pentameric Sec61-Sec62-Sec63 units form a translocation pore as it has been shown for the trimeric complex (5), then not more than 5% of all pores in the mammalian rough ER have a Sec-like structure.

What could be the function of the mammalian Sec61-62-63 complexes? Despite the clear differences between the cytosolic domains of this complex and the yeast Sec complex, one may

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2 H. Grau and E. Hartmann, unpublished information.
One should bear in mind that the majority of the Sec62 and the Sec63 were not found to be complexed. These populations could represent a pool that under appropriate conditions form Sec61-Sec62-Sec63 complexes. Alternatively, these molecules may perform functions while loosely associated with a subset of co-translational translocation sites. For example, they could recruit BiP molecules to resting trimeric Sec61 complexes in order to seal the pores, in alignment with a previous suggestion (20). They may also assist in the release of the translational pausing of polypeptides such as the apolipoprotein B (47).

The identification of a Sec-like complex in mammals again demonstrates the high degree of evolutionary conservation of the translocation machinery in the ER among eukaryotic organisms. However, the extent to which this structural similarity results in functional homology remains to be determined.

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