Membrane proteins of the endoplasmic reticulum (ER) may be localized to this organelle by mechanisms that involve retention, retrieval, or a combination of both. For luminal ER proteins, which contain a KDEL domain, and for type I transmembrane proteins carrying a dilysine motif, specific retrieval mechanisms have been identified. However, most ER membrane proteins do not contain easily identifiable retrieval motifs. ER localization information has been found in cytoplasmic, transmembrane, or luminal domains. In this study, we have identified ER localization domains within the three type I transmembrane proteins, ribophorin I (RI), ribophorin II (RII), and OST48. Together with DAD1, these membrane proteins form an oligomeric complex that has oligosaccharyltransferase (OST) activity. We have previously shown that ER retention information is independently contained within the transmembrane and the cytoplasmic domain of RII, and in the case of RI, a truncated form consisting of the luminal domain was retained in the ER. To determine whether other domains of RI carry additional retention information, we have generated chimeras by exchanging individual domains of the Tac antigen with the corresponding ones of RI. We demonstrate here that only the luminal domain of RI contains ER retention information. We also show that the dilysine motif in OST48 functions as an ER localization motif because OST48 in which the two lysine residues are replaced by serine (OST48ss) is no longer retained in the ER and is found instead also at the plasma membrane. OST48ss is, however, retained in the ER when coexpressed with RI, RII, or chimeras, which by themselves do not exit from the ER, indicating that they may form partial oligomeric complexes by interacting with the luminal domain of OST48. In the case of the Tac chimera containing only the luminal domain of RII, which by itself exits from the ER and is rapidly degraded, it is retained in the ER and becomes stabilized when coexpressed with OST48.

The endomembrane system of eukaryotic cells consists of a series of distinct membrane-bound organelles that communicate with each other by means of vesicular or tubular interactions (for review see Refs. 1 and 2). Despite extensive anterograde and retrograde traffic, cellular organelles maintain the characteristic protein and lipid composition necessary to execute their specific functions. Therefore, defined localization signals are required to establish the residency of a particular protein in a specific organelle or compartment (3). A paradigm for this model has been provided by the identification and characterization of a C-terminal KDEL motif. This motif is found at the C termini of several luminal proteins of the ER and serves to mediate the retention of resident proteins of the ER lumen through retrieval from post-ER compartments ER via a transport mechanism involving vesicular and tubular elements (4, 5). Another retention mechanism confers ER residency to membrane proteins that contain a cytoplasmically disposed C-terminal dilysine retention motif located close to the C terminus (6). This dilysine motif is recognized by coat protein I components, a membrane coat mainly concerned with retrograde transport from the Golgi apparatus back to the ER (5, 7–9).

Because the majority of ER membrane proteins do not have recognizable retrieval signals, it can be expected that other ER retention mechanisms exist. Misfolded or incompletely folded proteins may interact in the ER lumen with chaperones such as immunoglobulin heavy chain-binding protein, calnexin, or disulfide-isomerase, which by themselves carry retrieval signals (3, 10–12). Retention may also be achieved by the functional interactions of ER membrane proteins with an organized matrix of luminal ER proteins or with cytoskeletal elements via their cytoplasmic domains (3). Another type of retention mechanism involves interaction of membrane proteins with each other to form oligomeric complexes or even larger assemblies. This may be the case for membrane proteins of individual Golgi stacks (13–17), and it may also provide a mechanism to retain in the ER components of the translocation apparatus, as well as closely associated membrane components, including the OST complex (1, 18–21).

The mammalian oligosaccharyltransferase is composed of the four ER membrane proteins, ribophorin I and II (RI and RII), OST48, and DAD1, which form an oligomeric complex (22, 23). Newly synthesized polypeptides may be N-glycosylated by this OST complex by transferring oligosaccharides, which were preassembled on the membrane-bound dolichol-pyrophosphate lipid carrier, to asparagine residues in the context of Asn-Xaa- (Ser/Thr)-R. RI and OST48, and probably also RII, are type I transmembrane proteins. We have shown that the luminal domain of OST48 interacts with those of RI and RII and that the cytoplasmic domain of OST48 has affinity for the cytoplasmically exposed N-terminal tail of DAD1 (23). The OST complex interacts also with Sec61, the core component of the protein translocation apparatus (24), which also provides ribosomal binding sites (25). Results from experiments obtained over many years have demonstrated that components of

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the translocation apparatus, as well as those involved in the cotranslational modification of the newly synthesized polypeptides, are part of a proteinaceous network that confines these membrane proteins to the rough domain of the endoplasmic reticulum (18–20, 26, 27). Inclusion of proteins into this network would be an effective means of preventing their incorporation into vesicles that exit from the ER toward the Golgi apparatus. Our working hypothesis is that individual subunits of these oligomeric complexes carry by themselves ER localization motifs that function until the newly synthesized subunits are integrated into oligomeric complexes. Although OST48 contains a typical dilysine retrieval motif, RI, RII, and DAD1 do not carry established ER localization signals. We have previously shown that ER localization information in RII are contained independently in the cytoplasmic domain and in the transmembrane domain together with a short luminal flanking region. Here, we report that in RI only the luminal domain contains ER localization information and that replacement of the two lysine residues by serine in the dilysine retrieval motif of OST48 results in the expression of this OST subunit at the cell surface. Coexpression of OST48ss with RI or RII prevents the exit of OST48ss from the ER. This effect is most likely due to interactions between the luminal domain of OST48 with those of RI or RII. This conclusion is also supported by the finding that a Tac chimera where the luminal domain is replaced by that of RII is not only prevented from leaving the ER, but it becomes protected from degradation when coexpressed with OST48.

MATERIALS AND METHODS

Plasmid Construction—The rat RI cDNA was cloned in this lab by Harkin-Ort et al. (28). The dog OST48 cDNA was obtained by Dr. Greg Pirozzi (Cytogen Corp., Princeton, NJ). To construct plasmids containing the cDNAs encoding RI and OST48, the cDNAs were part of a proteinaceous network that confines these membrane proteins to the rough domain of the endoplasmic reticulum. The human Tac antigen cDNA was obtained by Dr. Greg Pirozzi (29). The human Tac antigen cDNA was obtained by Dr. Greg Pirozzi (29). The human Tac antigen cDNA was obtained by Dr. Greg Pirozzi (29). The human Tac antigen cDNA was obtained by Dr. Greg Pirozzi (29).

Table I

| Oligo number | Sequence (5’-3’) |
|--------------|-----------------|
| 1            | +               |
| 2            | +               |
| 3            | +               |
| 4            | +               |
| 5            | +               |
| 6            | +               |
| 7            | +               |
| 8            | +               |
| 9            | +               |
| 10           | +               |
| 11           | +               |
| 12           | +               |
| 13           | +               |

The sequences of oligonucleotides used for constructing OST48 and chimeras containing domains of RI and the Tac antigen. All sense strands are labeled “+”, whereas antisense strands are labeled “-”. Nucleotides typed in bold type indicate the EcoRI site. The underlined parts of oligo correspond to the Tac antigen sequence.

Table II

| Abbreviation of constructs | Oligo numbers |
|---------------------------|---------------|
| OST48                     | 1 and 2       |
| OST48ss                   | 1 and 3       |
| RI                        | 4 and 5       |
| Tac                       | 6 and 7       |
| T-I-T                     | 4, 8, 9, and 7|
| T-I-T                     | 6, 10, 11, and 7|
| T-I-T                     | 6, 12, 13, and 5|

*: For the structure of these constructs see Figure 1. 
#: These oligonucleotide numbers are those listed in table 1A.

of Dr. Herbert Samuels, New York University Medical Center, New York, NY. To construct plasmids containing the cDNAs encoding chimeras composed of domains of RI and the Tac antigen, I-T-T, T-I-T, and T-I-I (where I is RI, II is RII, and T is the Tac antigen), we generated cDNAs by using PCR for gene splicing by the overlap extension method as described previously (31). The primers used for PCR are described in Tables I and II. All three cDNAs have EcoRI sites. The transformation and preparation of plasmid DNA were carried out according to standard protocols. Schematic representation of the membrane disposition of all constructs are shown in Figure 1.

Cell Line and Culture Condition—HeLa cells were grown as monolayer cultures at 37 °C, 10% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 8% (v/v) fetal calf serum and 2 mM glutamine and penicillin/streptomycin. Transfection of cells, pulse-chase labeling, immunoprecipitation and Endo H treatment were done as described.2 Antibodies—The polyclonal rabbit anti-RI antibody was raised against RI purified from rat liver microsomes by SDS-polyacrylamide gel electrophoresis (32). The monoclonal anti-RI antibody was raised against SDS-polyacrylamide gel electrophoresis-purified rat RI. It detects an epitope located on the luminal domain of RI (33). The polyclonal antibody directed against OST48 was raised in rabbits against a fusion protein containing the luminal domain of dog OST48 fused at its C terminus with glutathione S-transferase using an expression vector developed by Ron and Dressler (34). To prepare the antibody that detects the Tac antigen, the mouse myeloma cell line 7G7B6 was propagated in BALB/c 3T3 mice (35), and the IgG fraction was purified from the ascites fluid by passing the fluid over a protein A-Sepharose column.

Immunofluorescence Microscopy—Transfected cells were grown on coverslips at 50–70% confluence (about 48 h after transfection), rinsed three times with 1 x cold phosphate-buffered saline, and then fixed with 3% paraformaldehyde for 20 min, followed by the same rinsing steps. The cells were treated with 0.2% Triton X-100 and incubated for 10 min if they had to be permeabilized. Permeabilized or nonpermeabilized cells were then incubated with 10 µg/ml of the primary antibody diluted in blocking solution in blocking solution. The monoclonal antibody α-RI was used at 1:200 dilution, the polyclonal antibody α-OST48 was used at 1:400 dilution, and the antibody against the Tac antigen, 7G7B6, was used at 1:100 dilution. After washing three times with blocking solution, the cells were incubated at 37 °C for 1 h with either fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG antibody or Texas Red-conjugated donkey anti-mouse IgG antibody dil-

2 Fu, J., Pirozzi, G., Sanjay, A. R. L., Levy, R., Chen, Y., DeLemos-Chiarandini, C., Sabatini, D. D., and Kreibich, G. (2000) Eur. J. Cell Biol., in press.
The chimera II-T-T is 516 amino acids long. OST48 is a mutated form of OST48 in which two lysine residues at positions 3 (amino acid 412) and 5 (amino acid 410) from the C-terminal end of the cytoplasmic domain of OST48 were replaced by serine. OST48ss is a mutated form of OST48 in which these two lysine residues were replaced by serine. B, RI (I), RII (II), and the Tac antigen (T) are represented as type I membrane proteins. The native proteins and the chimera are abbreviated such that the symbols I, II, and T correspond to the luminal, transmembrane, and cytoplasmic domains are separated by a hyphen. RI domains are represented by thick lines, and domains of the Tac antigen are represented by thin lines. The dotted line indicates the transmembrane domains. The numbers next to schematic representations of native OST48, of RI (I-I-I), or of the Tac antigen (T-T-T) correspond to the C termini of the luminal domains, the N-terminal ends of the cytoplasmic domains, and the C-terminal amino acids, thus defining the boundaries of the membrane proteins and of the chimera. The entire luminal domain of RII in the chimera II-T-T is 516 amino acids long.

RESULTS

The Luminal Domain of RI Contains ER Retention Information—RI is a type I transmembrane protein of the ER that does not contain identifiable ER localization signals, such as a C-terminally located dilysine motif. From previous studies we know that truncated forms of RI that consist of luminal portions of RI but are not anchored to the membrane are retained in the ER (36). When the membrane-anchored RI-Tac chimera I-T-T was expressed in HeLa cell, it was quantitatively retained in the ER as indicated by the typical ER staining patterns seen in immunofluorescence micrographs (Fig. 2B). On the other hand, the transmembrane and the cytoplasmic domains contain apparently no ER localization information because the Tac chimera T-T-T and T-I-T were expressed on the cell surface as detected by immunofluorescence of nonpermeabilized cells (Fig. 2, C and D). In the case of T-I-T, surface labeling could be detected even in permeabilized cells (Fig. 2D), suggesting that this chimera exits from the ER very efficiently, resulting in high concentrations of the chimera at the cell surface. It appears, therefore, that in RI only the luminal domain contains ER localization information. These results were confirmed by immunoprecipitation experiments. HeLa cells were transfected with cDNAs encoding the chimeras I-T-T (A and B), T-I-T (C and D), or T-T-I (E and F) and fixed with paraformaldehyde (A, C, and E) or fixed with paraformaldehyde and permeabilized with Triton X-100 (B, D, and F), followed by immunofluorescence staining with a monoclonal antibody against RI (α-I, A and B) or a monoclonal antibody against the Tac antigen (α-T, C-F). All samples were labeled using a Texas Red-conjugated donkey antiserum directed against mouse IgG. I-T-T was only expressed in the ER, whereas T-I-T and T-T-I were also found at the cell surface. The labeling conditions for these experiments, as well as those illustrated in Figs. 4 and 5 were chosen such that essentially only the overexpressed chimera and not the endogenous proteins were detected.

labeled for 30 min with [35S]methionine and then chased for 0 or 3 h. The chimeric proteins were immunoprecipitated using antibodies directed against RI (for I-T-T) or the Tac antigen (for T-I-T and T-T-I). Half of the samples were treated with Endo H.
were analyzed on a 12% SDS-polyacrylamide gel, followed by autoradiography. Arrowheads indicate the position of T-I-T and T-T-I that acquired Endo H resistance.

After a chase period of 3 h, I-T-T remained sensitive to the Endo H digestion (Fig. 3, lanes c and d), whereas T-I-T (lanes g and h) or T-T-T (lanes k and l) became at least partially Endo H-resistant (marked by an arrowhead). It is interesting to note that more of the chimera T-I-T became Endo H-resistant compared with T-T-I. These results are consistent with immunostaining experiments where cells expressing the chimera T-I-T showed much stronger surface immunofluorescence than those expressing T-T-I (Fig. 2). It appears, therefore, that the cytoplasmic domain of RI contains weak ER retention information. This was also seen when T-T-I was coexpressed with OST48s (see Fig. 6K).

The DilySine Motif at the Cytoplasmic Domain of OST48 Functions as an ER Localization Signal—OST48 is a type I ER transmembrane protein that contains a typical dilySine motif close to the C terminus. This motif serves as a retrieval signal in other type I ER transmembrane proteins (6). To test whether the dilySine motif in OST48 behaves indeed as an ER localization signal as predicted, HeLa cells were transfected with a cDNA encoding either the wild type OST48 or a mutant form of OST48 in which the two lysine residues were replaced by two serine residues (OST48ss) (Fig. 1A). As expected, in permealized cells we observed immunostaining of OST48 as a lace-like pattern characteristic of the rough endoplasmic reticulum (Fig. 4B), whereas nonpermealized cells remained unstained (Fig. 4A). In HeLa cells expressing OST48ss, surface labeling was detected in nonpermealized cells (Fig. 4C), and in addition, ER fluorescence was detected when the cells were permealized (Fig. 4D). These results demonstrated that the overexpressed wild type OST48 was retained in the ER, whereas OST48ss was not only found in the ER but also on the cell surface, thus confirming that the dilySine motif in OST48 does function as an ER localization signal, as was previously shown for the dilySine motif in Wbp1p, a yeast homolog of OST48 (37).

OST48ss Is Retained in the ER When Coexpressed with RI and RII—RI, RII, and OST48 together with DAD1 form an oligomeric complex that has oligosaccharyltransferase activity (22, 38). We have also shown that this oligomer is maintained by interactions between the luminal domain of OST48 with those of RI or RII (23). We therefore reasoned that when OST48ss is coexpressed with RI and/or RII, these subunits may form partial oligomeric complexes by interacting via their luminal domains. Two possibilities may be considered: either OST48ss, RI, and RII move together out of ER to the cell surface because by interacting with OST48ss the retention information of RI or RII are dominant. To test these two alternatives, OST48ss was coexpressed in HeLa cells with RI and/or RII, followed by communostaining. When expressed alone, OST48ss was found both at the cell surface (Fig. 5A) and in the ER (Fig. 5B), whereas coexpression of OST48ss and RI resulted only in ER staining of both proteins (Fig. 5, A–D) or the chimera containing both luminal and transmembrane domains of RI and RII and the cytoplasmic domains of the Tac antigen (I-T-T; Fig. 6, A–D) or the chimera containing both luminal and transmembrane domains of RI and the cytoplasmic domain of the Tac antigen (II-T-T) (data not shown).2 We have shown that the chimera I-T-T and T-T-I are expressed at the cell surface (Fig. 6, C and E, respectively). When coexpressed with OST48ss either one of the RI-Tac chimera will exit from the ER and they, as well as OST48ss can be detected at the cell surface (Fig. 6, E, G, I, and K). These results are best explained by assuming that the luminal domain of OST48ss interacts with those of RI or RII, as was recently demonstrated (23), and that these partial oligomeric complexes are retained in the ER when one of the partners has ER retention information.

II-T-T Is Retained and Stabilized in the ER by Interacting with OST48—As described before, when the chimera II-T-T was expressed in HeLa cells, it exited from the ER and was found at the cell surface, as well as in a lysosomal compartment.2 It was rather quickly degraded (t1/2 ~ 2 h), and the addition of leupeptin, an inhibitor of lysosomal proteases, largely prevented its degradation. Based on the coexpression experiments shown above, it was expected that II-T-T is retained by OST48 in the ER through the interaction between their luminal domains. We therefore transfected HeLa cells with the Tac chimera II-T-T alone or II-T-T together with OST48, followed by pulse-chase labeling and immunoprecipitation using an antibody directed against the luminal domain of RII. Aliquots of the immunoprecipitates were then treated with Endo H.

As shown in Fig. 7 (lane a), immediately after the pulse period, the major band immunoprecipitated with the anti-RII

\[ \text{OST48ss (α-OST48)} \]

Non-Permeabilized Permeabilized

\[ \text{OST48} \]

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antibody corresponds to the N-glycosylated form of the Tac chimera II-T-T (II-T-T*), although a small fraction is made as an unglycosylated protein (II-T-T). In addition, two faint bands of about equal intensity (lane a) corresponding to the N-glycosylated (RII*), and unglycosylated forms of the endogenous RII (RII) can be seen. Digestion of half of this immunoprecipitate with Endo H (lane b) results in a shift of the glycosylated form of RII into a band with higher electrophoretic mobility, corresponding to the nonglycosylated form (RII). A similar effect was observed when the II-T-T chimera was digested with Endo H (II-T-T in lane b) results in a shift of the glycoylated form of RII into a band with higher electrophoretic mobility, corresponding to the nonglycosylated form (RII). After a chase period of 3 h, the intensity of the bands corresponding to the endogenous RII remains unaltered (compare RII in lane d with that in lane d), whereas the intensity of the band corresponding to the chimera II-T-T in lane d is less than 15% compared with that in lane b. The faint and rather diffused band with significantly lower electrophoretic mobility (II-T-T* in lanes c and d) corresponds to II-T-T molecules that have exited from the ER and received modification of the N-linked oligosaccharide that rendered it insensitive to Endo H digestion. When II-T-T and OST48 were coexpressed in HeLa cells, the band corresponding to II-T-T* carrying a complex oligosaccharide is no longer seen (lanes g and h), indicating that the chimera no longer leaves the ER. Instead the protein carrying a high mannose oligosaccharide remains Endo H-sensitive even after a chase period of 3 h (lane h). Noteworthy is also the fact that the chimera is not only retained in the ER, but it becomes stabilized; the ratio of the intensity of the radioactive bands corresponding to the endogenous RII and the chimera II-T-T immunoprecipitated immediately after the pulse (0 h; lane f) is similar to that obtained after a chase period of 3 h (lane h). It appears, therefore, that through interactions of the luminal domains of RII and OST48 the chimera, II-T-T was not only retained in the ER, but it was also protected from proteolytic degradation.

FIG. 5. OST48ss is retained in the ER when coexpressed with RI. HeLa cells were transfected with cDNA encoding OST48ss (A and B), followed by immunostaining as described in the legend to Fig. 4. Cells were also cotransfected with cDNAs encoding OST48ss and RI (C–F) and fixed (C and E), or they were fixed and in addition permeabilized (D and F) as described before. For double immunofluorescence labeling, antibodies directed against OST48 or RI were used (for details, see "Materials and Methods"). Results shown in C–F demonstrate that OST48ss is retained in the ER when coexpressed with RI.

FIG. 6. OST48ss is retained in the ER when coexpressed with the chimera I-T-T but not with the chimeras T-I-T or T-T-I. HeLa cells were cotransfected with cDNAs encoding OST48ss and I-T-T (A–D), OST48ss and T-I-T (E–H), or OST48ss and T-T-I (I–L). They were then fixed (A, C, E, G, I, and K) or fixed and permeabilized (B, D, F, H, J, and L) followed by double immunofluorescence labeling as described under "Materials and Methods." OST48ss was retained in the ER (B) when coexpressed with the chimera I-T-T (A–D) but not when the chimeras T-I-T (E–H) or T-T-I (I–L) were coexpressed.

DISCUSSION

This study is mainly concerned with an analysis of the polypeptide domains of RI and OST48 that contribute to their retention in the ER. Although native subunits, modified polypeptides, or Tac chimeras were overexpressed, the results obtained are expected to be functionally relevant with regard to the biosynthesis of the OST complex, because before integration into oligomeric assemblies, subunits of the OST complex exist as individual molecules, and similar retention mechanisms are expected to act on them as on overexpressed subunits and chimeras containing specific domains of the OST subunits. The retention of the OST complex in the rough portion of the ER is further complicated by the fact that in addition to retention mechanisms that affect the individual subunits or the oligomer, this complex is known to coassemble with the Sec61p complex (24), which is part of an oligomeric array that extents throughout the rough portion of the ER, thus interconnecting translocation sites and segregating them from the smooth ER.

ER Retention of Oligosaccharyltransferase Complex Subunits
after transfection, cells were metabolically labeled with [35S]methionine.

Histagged II-T-T alone (lanes a–d) or together with OST48 (lanes e–h). 44 h after transfection, cells were metabolically labeled with [35S]methionine for 30 min at 37°C and then chased for 0 (lanes a, b, e, and f) or 3 h (lanes c, d, g, and h). Labeled proteins were immunoprecipitated with a polyclonal antibody against RII, and half of the samples were subjected to Endo H treatment, followed by SDS-polyacrylamide gel electrophoresis and autoradiography (for details, see legend to Fig. 3). The glycosylated and nonglycosylated forms of endogenous RII are indicated as RII* and RII, respectively (labeled at left side). Similarly, the II-T-T* and II-T-T (labeled at right side) correspond to the glycosylated and nonglycosylated forms of chimera II-T-T, respectively. The symbol II-T-T** indicates that the modified II-T-T chimera is resistant to Endo H treatment.

(1, 18, 39). Two retrieval signals, the KDEL (4, 40), and the dileucine motif (41, 42), located in the ER lumen and the cytoplasm, respectively, are well characterized. In addition, proteins could be retained in the ER through interactions with chaperones (12, 43–45) or cytoskeletal elements (46), forming large aggregates by themselves (47) or by being incorporated into a proteinaceous network (1, 18, 39). By applying immunofluorescence microscopy on transfected cells and immunoprecipitation of labeled proteins, we have begun to characterize domains within the individual OST subunits that prevent them from leaving the ER.

The function of the dileucine retrieval signal found in Wbpl, a yeast homolog of OST48, had been previously investigated (37). We hypothesize that after synthesis, some of the unassembled OST48 molecule may exit from the ER, and they would be retrieved from a post-ER compartment via coat protein I-coated vesicles. The retention mechanisms that affect the luminal domain of RI or the cytoplasmic and transmembrane domain of RII remain to be determined. It is very unlikely, however, that in normal cells, where the OST subunits are expressed in the proper stoichiometric ratio, RII or RII are retained in the ER by retrieval from post ER compartments. They do not acquire glycosylation patterns typical for post-ER compartments (41, 48), despite the fact that both membrane glycoproteins can acquire complex N-linked oligosaccharides and O-linked sugars when exposed to Golgi glycosyl transferases (49). When OST subunits or chimeras such as I-T-T are overexpressed compared with OST48, it is even less likely that they are retrieved from post-ER compartments, because only a small fraction would be able to oligomerize with OST48, which is the only AST subunit that carries an established ER retrieval signal. We had previously investigated the retention of luminal domains of RI by expressing in HeLa cells two C-terminally truncated variants of RI, R167 and R1322, where R1465 is still membrane anchored but lacks part of the cytoplasmic domain, and R1322 consists of a partial luminal domain of RI (36). Both R167 and R1322 were rapidly degraded by a nonsolomosal mechanism. Interestingly, R1322 was degraded in a biphasic fashion and was not found to be associated with immunoglobulin heavy chain-binding protein (36). Recent studies have provided evidence that this so-called nonsolomosal ER degradation is in fact mediated by cytoplasmically located proteasomes (50, 51). It is possible that in an unassembled monomer, such as I-T-T, the luminal domain functions as a true retention signal even after partial complexes are formed with OST48ss. It is more likely, however, that the luminal domain in I-T-T and also in RI is not properly folded because it lacks the other subunits of the OST complex as interacting partners. In this state of incomplete folding they are expected to be recognized by and bound to the transmembrane chaperone calnexin that is known to interact with incompletely assembled ER glycoproteins (12, 43, 44). Alternatively, they may form aggregates by themselves that are large enough to be excluded from transport vesicles budding off the ER.

Kelleher and Gilmore (22) showed that RI, RII, OST48, and DAD1 can be isolated as an oligomeric complex, and we have determined the interacting domains within the OST subunits. Specifically we found that the luminal domain of OST48 interacts with those of RI and RII (23). It may therefore be expected that through this type of intermolecular interactions, polypeptides related to the OST subunits may be indirectly retained in the ER. It was indeed found that when OST48ss was coexpressed with RI or RII it was no longer able to exit from the ER. One can therefore assume that as soon as OST48 is integrated into the OST complex, it is retained in the ER through interactions with the luminal domains of RI and RII. As may be expected, the formation of links between OST subunits is steps toward stabilization of the individual subunits, which protects them from “ER degradation” of unassembled subunits (52). This is in agreement with a demonstration that a mutation in the DAD1 subunit leads not only to the degradation of DAD1, but it also destabilizes the other three subunits (53). We have also shown that the chimera II-T-T whenever expressed by itself is able to exit from the ER and it accumulates at the cell surface and in lysosomes where it is degraded.2 However, when this chimera is coexpressed with OST48, both polypeptides are retained in the ER and protected from lysosomal degradation.

It is interesting to note that, in contrast to the native RII, which is found in about equimolar amounts as N-glycosylated and nonglycosylated forms (Ref. 54 and Fig. 7, lane a), the newly synthesized Tac chimera II-T-T was almost quantitatively N-glycosylated. This finding cannot be easily explained if one assumes that N-glycosylation is a strictly cotranslational process, in which growing nascent polypeptide chains are presented to the luminal oriented oligosaccharide transferase in an identical fashion, irrespective of the type of transmembrane or cytoplasmic domain of the mature polypeptide. Alternatively, N-glycosylation of the growing nascent chain may not occur in a strictly cotranslational manner (55), which is also suggested by the fact that the acylated tripeptide Asn-Tyr-Ser can be posttranslationally N-glycosylated (38, 56). The extensive N-glycosylation of Asn41 in II-T-T may therefore reflect their extended accessibility to the N-glycosylation machinery, possibly because of their inability to become incorporated into the oligomeric structure associated with the translocation apparatus in the rough endoplasmic reticulum (see also Ref. 49).

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