Targeting to the Endoplasmic Reticulum in Yeast Cells by Determinants Present in Transmembrane Domains*

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The transmembrane domains (TMDs) of many type I integral membrane proteins contain determinants that cause localization in the endoplasmic reticulum (ER) in mammalian cells by an unknown mechanism. Here we show that the yeast ER localization machinery recognizes determinants in TMDs that are very similar to those identified previously in mammalian cells. These determinants are recognized in post-ER compartments and recycled back to the ER, thus acting as ER retrieval signals. Moreover determinants in TMDs are inefficiently sorted in several previously characterized yeast mutants with defects in the ER retrieval machinery. Similar ER retrieval signals are also recognized in the TMDs of polytopic integral membrane proteins, apparently by the same sorting machinery. The isolation of new mutants defective in sorting of membrane determinants might provide a better understanding of the molecular mechanisms involved in this process.

In many type I and II transmembrane proteins, transmembrane domains (TMDs)1 are composed of approximately 20 hydrophobic amino acid residues, which form a helix spanning the lipid bilayer (1). Besides their structural role as membrane spanning domains, TMDs of type I transmembrane proteins can contain complex determinants (hereafter referred to as membrane determinants), which play a key role in the assembly and intracellular transport of many oligomeric membrane receptors. For example, specific interactions between TMDs are essential for the assembly of major histocompatibility complex class II molecules, CD8 molecules, the T-cell receptor, and the human high affinity receptor for immunoglobulin E (FceRI) (2–6). For several of these multisubunit receptors (T-cell receptor, CD8, FceRI, B-cell receptor), membrane determinants also ensure retention in the endoplasmic reticulum (ER) of unassembled chains, thus participating in quality control at the level of the ER (3, 6–10). It is not known whether the observed ER localization results from continuous recycling from post-ER compartments, or from true ER retention. A usual feature of membrane determinants for ER retention is the presence of one or several hydrophilic residues within the hydrophobic TMD.

These residues can be potentially charged residues, strongly hydrophilic residues, or several weakly hydrophilic residues (9, 10). The introduction of one or several polar amino acid residues in the hydrophobic segment of a type I transmembrane protein normally transported to the cell surface is sufficient to cause its localization in the ER. Strongly hydrophilic residues are more efficient than weakly hydrophilic residues, and their effect is strongest when they are localized toward the middle of the TMD (9). Longer and more hydrophobic transmembrane domains require the insertion of more hydrophilic residues to be able to cause ER retention (11).

Similar membrane determinants are also involved in the ER retention of the precursors of glycosphatidylinositol (GPI)-anchored proteins. The precursors of GPI-anchored proteins are generally composed of an extracellular domain, a hydrophobic membrane-anchoring segment (referred to as a TMD) and a very short or no cytoplasmic domain. Shortly after their synthesis, precursors are cleaved at a luminal site near the TMD and attached to a GPI anchor. When the cleavage site is mutated, the precursor is retained in the ER by signals contained in its TMD (12). This system participates in the quality control of GPI-anchored proteins by allowing transport to the cell surface of mature GPI-anchored proteins while unprocessed precursors are selectively retained in the ER.

In many cases Saccharomyces cerevisiae has provided a convenient model to analyze the cellular mechanisms regulating intracellular traffic, but to date it is not clear if yeast cells make use of sorting determinants in TMDs analogous to those identified in mammalian cells. It has been shown that the TMD of Sec12p causes ER localization by continuous retrieval to the ER, and this was shown to be dependent on Rer1p and coatomer (13). On the other hand, the TMD of Ufe1p was shown to cause ER localization in a Rer1p-independent manner (14). Here we characterize extensively membrane determinants for ER localization in yeast cells, and show that they are very similar to those characterized previously in mammalian cells. Localization in the ER is achieved by continuous retrieval from post-ER compartments, and is affected in Δrer1 and other ER retrieval mutants. The same sorting machinery can also be used to achieve selective ER localization of polytopic membrane proteins.

MATERIALS AND METHODS

Strains, Media, and Reagents—Yeast media have been described (17). Yeast strains used in this study are listed in Table I. Note that the endogenous SUC2 gene is disrupted in all cells used for the expression of invertase fusion proteins. Unless otherwise specified, all mutants used correspond to the allele –1 (sec27 for sec27–1, etc.).

The original SUC2/GAS1 fusion was obtained from C. Nuoffer and H. Riezman by fusing the sequence coding for the last 159 amino acid residues of Gas1p at the C terminus of the invertase (SUC2) gene. The chimeric gene is under the control of a constitutive PRC1 promoter. The

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1 The abbreviations used are: TMD, transmembrane domain; GPI, glycosphatidylinositol; ER, endoplasmic reticulum; COP, coat protein; endo H, endoglycosaminidase H; pep4, vacuolar protease PrA; PAG, polyacrylamide gel electrophoresis.

‡ P. Cosson, unpublished observations.
ER Retrieval Signals in Transmembrane Domains

### RESULTS

**The Transmembrane Domain of the Gas1p Precursor Ensures Its Localization in the ER**

The Gas1 protein is synthesized in the endoplasmic reticulum of yeast cells as a membrane-bound precursor with a TMD and no cytoplasmic domain. Shortly after its synthesis, the precursor is cleaved at a site near the TMD, and the hydrophobic extension is replaced with an asparaginyl-containing glycopeptidoglycan that anchors the protein to the membrane (18). During transport of GPI-anchored Gas1p to the cell surface, maturation of Gas1p in the Golgi apparatus due to extensions of N-linked and O-linked carbohydrate chains decreases its mobility in SDS-PAGE, from 105 to 125 kDa (Refs. 18 and 27; see also Fig. 1). When the anchor attachment site (asparagine in wild-type Gas1p) is mutated to a glutamine residue (Gas1Q), the resulting protein retains its TMD and is localized in the ER (Ref. 28; see also Fig. 1C). As mentioned above, hydrophilic residues are a usual feature of membrane determinants for ER localization in mammalian cells, and the TMD of Gas1p contains several serine and threonine residues. When several of these (three serine residues and one threonine) are mutated to leucine, the resulting Gas1Q[L] protein retains its TMD, but is transported out of the ER, albeit less efficiently than the wild-type Gas1p (Ref. 27; see also Fig. 1). This suggests that the TMD of Gas1p plays a critical role in retaining the uncleaved precursor in the ER, and that hydrophilic residues are elements of the membrane determinant for ER localization.

**ER Retrieval of Invertase-Gas1Q Chimeras**—To test whether the transmembrane domain of Gas1p can act as a determinant for localization in the ER, the C-terminal segment of Gas1Q or of Gas1Q[L] was transferred to the COOH terminus of invertase (Inv-Gas1Q[ST] and Inv-Gas1Q[L], respectively; Fig. 2A).

### Table I

**Yeast strains**

| Strain | Genotype | Origin |
|--------|----------|--------|
| PC167  | MATa, ura3, leu2, his4, bar1, gas1::LEU2, [GAS1WT::URA3]CEN | H. Riezman |
| PC183  | MATa, ura3, leu2, his4, bar1, gas1::LEU2, [GAS1Q::URA3]CEN | H. Riezman |
| PC184  | MATa, ura3, leu2, his4, bar1, gas1::LEU2, [GAS1QL::URA3]CEN | H. Riezman |
| PC142  | MATa, ura3, leu2, his3, trp1, lye2, gas1::LEU2, suc249, [Inv-Gas[ST]::URA3]CEN | This study |
| PC143  | MATa, ura3, leu2, his3, trp1, lye2, gas1::LEU2, suc249, [Inv-Gas[L]::URA3]CEN | This study |
| PC188  | MATa, ura3, leu2, his3, trp1, lye2, gas1::LEU2, suc249, Δpеп4::LEU2, [Inv-Gas[ST]::URA3]CEN | This study |
| PC189  | MATa, ura3, leu2, his3, trp1, lye2, gas1::LEU2, suc249, Δpеп4::LEU2, [Inv-Gas[L]::URA3]CEN | This study |
| PC182  | MATa, Δεр1::TRP1, ura3, leu2, his3, trp1, ade2, suc249, [Inv-Gas[ST]::URA3]CEN | This study |
| PC166  | MATa, ret−1−1, ura3, leu2, his3, trp1, suc249, [Inv-Gas[ST]::URA3]CEN | This study |
| PC161  | MATa, ret−2−1, ura3, leu2, his3, lye2, suc249, [Inv-Gas[ST]::URA3]CEN | This study |
| PC163  | MATa, ret−3−1, ura3, leu2, his3, trp1, suc249, [Inv-Gas[ST]::URA3]CEN | This study |
| PC202  | MATa, sec23−1, ura3, leu2, his3, lye2, suc249, [Inv-Gas[ST]::URA3]CEN | This study |
| FLY276 | MATa, ura3, his3, trp1, ade2, suc249, ste2::LEU2, STE2-P290D::URA3 | This study |
| FLY272 | MATa, Δεр1::TRP1, ura3, leu2, his3, trp1, ade2, suc249, ste2::LEU2, STE2-P290D::URA3 | This study |
| FLY371 | MATa, ret−1−1, ura3, his4, trp1, leu2, ste2::LEU2, STE2-P290D::URA3 | This study |
| FLY372 | MATa, ret−3−1, ura3, leu2, his4, ade2, ste2::LEU2, STE2-P290D::URA3 | This study |
| FLY373 | MATa, sec23−1, ura3, leu2, his4, bar1, ste2::LEU2, STE2-P290D::URA3 | This study |
| RH311–3D | MATa, ura3, leu2, trp1 | H. Riezman |

### Figure 2A

**ER Retrieval of Invertase-Gas1Q Chimeras**—To test whether the transmembrane domain of Gas1p can act as a determinant for localization in the ER, the C-terminal segment of Gas1Q or of Gas1Q[L] was transferred to the COOH terminus of invertase (Inv-Gas1Q[ST] and Inv-Gas1Q[L], respectively; Fig. 2A).
For all the constructs used here, the main characteristic of the TMD is indicated in brackets ([ST]) for a TMD containing serine and threonine residues, [L] for a TMD where they are mutated to leucine residues, etc.). Upon transport to the Golgi apparatus, the N-linked oligosaccharides attached to invertase are processed extensively by the Golgi mannosyltransferases and secreted invertase migrates as a very high molecular mass heterogeneous smear on SDS-polyacrylamide gel. To characterize the glycosylation state of the invertase-Gas1Q fusion proteins, yeast cells expressing either Inv-Gas1Q[ST] or Inv-Gas1Q[L] were pulse-labeled for 10 min with Trans-35S-label, and equal aliquots of cells were harvested after 0 or 1 h of chase. The immunoprecipitated fusion proteins were split into equal aliquots, and one aliquot was treated with endo H to remove N-linked sugars. Before the chase, Inv-Gas1Q[ST] migrated as a 130-kDa band (Fig. 2B, lane 1), and after a 1-h chase, it showed only a slight increase in apparent molecular mass, as observed commonly for invertase when it is retained in the ER (Fig. 2B, lane 2). Endo H treatment removed N-linked sugars and converted the 130-kDa band to a 95-kDa band (Fig. 2C, lanes 1 and 2), the expected size of the unglycosylated fusion protein. Inv-Gas1Q[L] also migrated as a 130-kDa band after the pulse, although a fraction migrated as a very high molecular mass species (>200 kDa) (Fig. 2B, lane 3). After the chase, the protein migrated as a heterogeneous smear at 130–200 kDa (Fig. 2B, lane 4). Upon endo H treatment, a doublet at 95 and 115 kDa was observed after the pulse (Fig. 2C, lane 3), while after the chase a unique 55-kDa band was seen (Fig. 2C, lane 4). Thus, during the chase, the Inv-Gas1Q[L] undergoes proteolytic cleavage and a fragment corresponding to the size of wild-type invertase is generated. To check if this proteolytic processing took place in the vacuole, we expressed the invertase-Gas1Q fusion proteins in a protease-deficient Δpep4 strain. The PEPl gene encodes the vacuolar hydrolase proteinate A (PrA), which is required for the activation of several protease zymogens within the vacuole. The proteolytic processing of the Inv-Gas1Q[L] was almost completely abolished in Δpep4 cells (Fig. 2, lanes 7 and 8). In these cells, the fusion protein accumulated as a very high molecular mass species (>200 kDa), and upon endo H treatment migrated as a 115 kDa band. These results demonstrate that, following transport through the Golgi apparatus, Inv-Gas1Q[L] reaches the vacuole where it is processed by vacuolar pro- teases. This is similar to the intracellular fate of other previously described membrane-bound invertase fusion proteins, which are also transported to the vacuole when they exit from the early secretory pathway (19).

The conversion to a 115-kDa form upon transport out of the ER presumably results from the elongation of endo H-resistant O-linked sugars in the Golgi apparatus. Indeed the Gas1 protein has been reported to be heavily O-glycosylated and many potential O-glycosylation sites are found in the membrane-proximal segment of Gas1p (18). Consistent with this interpretation, the 115-kDa form exhibits α1,3-mannose sugars (see below), indicating that it has reached late Golgi compartments.

Detailed analysis of the processing of N-linked sugars can provide information on the intracellular compartments reached by a glycoprotein. Addition of α1,6-mannose occurs in an early Golgi compartment, while elongation of α1,6-mannose sugar chains and addition of α1,3-mannose occurs in later Golgi compartments. After a 1-h chase, both Inv-Gas1Q[ST] and Inv-Gas1Q[L] could be quantitatively reprecipitated with an anti- serum to α1,6-mannose (Fig. 3B), indicating that both fusion proteins get access to an early Golgi compartment. However, only Inv-Gas1Q[L] acquired α1,3-mannose sugars, indicating that it is transported to later compartments of the Golgi apparatus. In sec18 mutant cells at 37 °C, vesicular transport from the ER to the Golgi was blocked and no acquisition of α1,6- or α1,3-mannose was observed (data not shown). This confirms that the addition of these sugars is due to vesicular transport of invertase-Gas1Q fusion proteins to the Golgi apparatus.

Together, these experiments demonstrate that Inv-Gas1Q[ST] is retained in early secretory compartments (ER

Fig. 1. A, schematic representation of the precursor of Gas1p. The cleavage/attachment site (N for wild type, Q for mutants) is indicated, as well as the sequence of the membrane anchor. B–D, intracellular processing of Gas1p and mutants of Gas1p. Cells expressing wild-type Gas1p (B), Gas1Qp (C), or Gas1QLp (D) were metabolically labeled for 10 min at 30 °C with [35S]methionine and chased for 0 or 1 h, before lysis and immunoprecipitation of Gas1p. Upon glycosylation in the Golgi apparatus, Gas1p is converted from a precursor (p) to a mature (m) form.

Fig. 2. A, the sequence coding for the last 159 amino acid residues of Gas1p was fused at the C terminus of the invertase (SUC2) gene. The sequence of the wild-type ([ST]) or mutated ([L]) membrane anchor is indicated. B and C, intracellular processing of invertase-Gas1 fusion proteins. Wild-type cells (lanes 1–4) or Δpep4 cells (lanes 5–8) were transformed with plasmids coding for Inv-Gas1Q[ST] (lanes 1 and 2, and lanes 5 and 6), or Inv-Gas1Q[L] (lanes 3 and 4, and lanes 7 and 8). The cells were metabolically labeled for 10 min and chased for 0 or 1 h before lysis and immunoprecipitation with an antiserum to invertase and analysis of Inv-Gas1Q by SDS-PAGE, with (C) or without (B) prior treatment with endo H.

ER Retrieval Signals in Transmembrane Domains

33275
and/or cis-Golgi) but they do not allow to determine precisely the site of accumulation. Inv-Gas1Q[ST] could accumulate in an early Golgi compartment, or it could be retrieved from this compartment back to the ER. To distinguish between these two possibilities, we analyzed the intracellular localization of Inv-Gas1Q[ST] by subcellular fractionation. Metabolically labeled cells expressing Inv-Gas1Q[ST] were lysed, and the supernatant of a 500 × g spin was fractionated by velocity sedimentation on a sucrose gradient. The presence of Golgi (Emp47p) and ER (Wbp1p) markers in the gradient fractions was analyzed by immunoblotting (Fig. 4). Inv-Gas1Q[ST] was detected by immunoprecipitation with an antibody to invertase, and one aliquot was further precipitated with antibodies to α1,6-mannose (Fig. 4a). Inv-Gas1Q[ST] cofractionated with Wbp1p (fractions 8–11), and was mostly absent in fractions containing Emp47p (fractions 2–5). Inv-Gas1Q[ST] from ER fractions was quantitatively precipitated with antibodies to α1,6-mannose (Fig. 4b) but not with antibodies to α1,3-mannose (data not shown), demonstrating that after acquisition of α1,6-mannose in early Golgi compartments, Inv-Gas1Q[ST] is retrieved selectively from early Golgi compartments to the endoplasmic reticulum.

Membrane Determinants for ER Retrieval Are Similar in Yeast and Mammalian Cells—To ascertain that the determinant for ER retrieval of Inv-Gas1Q was solely comprised in its TMD, we constructed a chimeric protein where only the TMD was derived from Gas1, while the lumenal domain was derived from invertase (A), α1,6-mannose (B), or α1,3-mannose (C).

![Fig. 3. Mannose modifications of invertase-Gas1Q fusion proteins.](image)

The fusion protein was immunoprecipitated with an antibody to invertase, eluted from the beads, and reprecipitated with antibodies to invertase (A), α1,6-mannose (B), or α1,3-mannose (C).

![Fig. 4. Intracellular distribution of Inv-Gas1Q[ST] analyzed by velocity sedimentation on sucrose gradients.](image)

Yeast cells expressing Inv-Gas1Q[ST] were metabolically labeled for 20 min, and then chased for 40 min before homogenization. The cleared lysate was loaded onto a sucrose gradient and spun. Gradient fractions were collected (top, fraction 1; bottom, fraction 10; pellet, fraction 11), and an aliquot was analyzed by SDS-PAGE and immunoblotting using antibodies to an ER marker (Wbp1p) or a Golgi marker (Emp47p). Each fraction was then immunoprecipitated with an antibody to invertase. One aliquot of the immunoprecipitated material was eluted from the beads and reprecipitated with antibodies to α1,6-mannose (Δ). For each marker analyzed, the relative intensity indicates the amount of signal relative to the maximum.

**ER Retrieval Signals in Transmembrane Domains**

To better define the membrane determinants for ER retrieval in yeast cells, we introduced various polar residues in the TMD of invertase-Gas1Q constructs. The presence of a single hydrophilic residue in the TMD of Inv-GasQ ensured its localization in the ER (Fig. 5), but with variable efficiency: aspartic acid (D) was more efficient than arginine (R), and glutamine (Q) was the least efficient. This is reminiscent of experiments in mammalian cells, where aspartic acid residues in TMDs were found to be more effective than arginine residues at causing ER retention (9), while glutamine residues were even less effective. When the TMD was lengthened by the addition of two hydrophobic residues ([long]), only an aspartic acid in the TMD could still cause ER localization, while constructs with an arginine or a glutamine residue in the TMD were transported efficiently to the vacuole (Fig. 5). Thus, when the TMD is longer, only the most potent membrane determinants ([ST] or [D]) can still cause ER localization. This is similar to observations in mammalian cells, where it was shown that longer TMDs require the insertion of more hydrophilic residues to cause ER retention (11). Thus, by all criteria tested here, membrane determinants recognized by the ER localization machinery are very similar in yeast and in mammalian cells.

**Rer1p and the Coatomer Complex Are Essential for Efficient ER Retrieval of Proteins Bearing Membrane Determinants** —To determine the role of various gene products in the sorting and ER retrieval of membrane determinants, we analyzed the intracellular transport of Inv-Gas1Q[ST] in various mutant cells. Coatomer is a protein complex composed of seven subunits, α-, β-1, β-2, γ, δ, ε and ε-COP, which forms a coat around vesicles budding from the Golgi apparatus (29). In Saccharomyces cerevisiae, coatomer mutants are defective in retrograde transport of dicyanine-tagged proteins from the Golgi to the ER at 30 °C (22, 30). All the experiments presented below were done at 30 °C to avoid the complete block of transport observed in many mutants at 37 °C. No vacuolar processing of Inv-Gas1Q[ST] was observed in any of the mutants tested (sec7, sec12, sec13, sec14, sec16, sec17, sec18, sec19, sec20, tip20, sec21, sec23, ret1, ret2, ret3; Fig. 6A and data not shown). We also observed no accumulation of invertase activity at the cell surface (data not shown).
ER Retrieval Signals in Transmembrane Domains

Fig. 5. The length of the transmembrane domain modulates the effect of membrane determinants for ER retrieval. Inv-GasQ fusion proteins with a TMD of 18 or 20 (long) amino acid residues were constructed and various hydrophilic residues introduced in their TMD. In these constructs a cytoplasmic domain was also added, composed of the sequence RRSMEQLILSEEDLN, to make certain that the TMD was positioned correctly within the membrane. The vacuolar processing of Inv-GasQ mutants was analyzed by SDS-PAGE following endo H treatment, as described in the legend to Fig. 2A. No noticeable acquisition of 1,3-mannose was evident in other mutants tested (sec21–1, sec21–2, sec23, sec25, sec18, sec27; Fig. 6B and data not shown), although a minor effect could easily be overlooked. Upon subcellular fractionation, the bulk of the Inv-Gas1Q[ST] was still present in the ER in ret1–1 cells, and a fraction of it could be reprecipitated with antibodies to α,1,3-mannose (data not shown) suggesting that in these cells at least a fraction of Inv-Gas1Q[ST] was transported further into the Golgi apparatus before being retrieved to the ER. We cannot, however, exclude the possibility that acquisition of α,1,3-mannose was due to a partial redistribution of the α,1,3-mannosyltransferase from the late Golgi compartments to the cis-Golgi or the ER. It is noteworthy, however, that all the mutants exhibiting this putative defect in ER retrieval of Inv-Gas1Q[ST] are mutants of coatomer subunits. The coatomer complex has already been implicated in ER retrieval of dilyosine-tagged proteins, and this result suggests that the same retrieval pathway is followed by proteins bearing membrane determinants.

We also tested the role of Rer1p, another protein previously implicated in ER retrieval in yeast cells (13, 32–34). In Δrer1 mutant cells, Inv-Gas1Q[ST] was transported to the vacuole where it was processed (Fig. 7). Other invertase-Inv-GasQ fusion proteins (Inv-GasQ[R], -[D], and -[Q]) were also transported efficiently to the vacuole in Δrer1 mutant cells (data not shown). The retention defect was corrected by transforming the cells with a wild-type copy of RER1 (Fig. 7). Thus, Rer1p is involved in ER retrieval of membrane determinants present in type I transmembrane proteins.

Membrane Determinants for ER Retrieval in Polytopic Membrane Proteins—Polytopic membrane proteins, exhibiting several membrane-spanning segments, can also be localized in the early secretory apparatus. For example, the KDEL receptor cycles between the ER and early Golgi compartments, and mutating a single aspartic acid residue to leucine in the seventh TMD abolishes its retrieval to the ER (35). However it remains to be seen if a single polar residue in one TMD can cause ER localization of a polytopic membrane protein. To test this, we introduced an aspartic residue at an equivalent position in the seventh TMD of Ste2p (P290D). Ste2p is normally expressed at the surface of MATa yeast cells and is essential for mating. The wild-type STE2 was deleted in MATaΔste2 yeast cells expressing Ste2p-P290D were grown on YPD plates and replica-plated to a lawn of MATα cells. After 6 h of mating at 30 °C, cells were replica-plated to SD plates selective for the growth of diploid cells. Only MATα cells deficient in ER retrieval of Ste2p-P290D were capable of mating with MATα cells and of generating diploids.
defects in ER retrieval (ret1, ret2, ret3, sec27, sec20, tip20; Fig. 8 and data not shown). No significant mating was observed in sec23, sec13, sec16, and sec17 mutant cells (Fig. 8 and data not shown). These results strongly suggest that a single polar amino acid residue can cause ER localization of a polytopic membrane protein if it is placed in a TMD at an appropriate position, and that this sorting is dependent on Rer1p and the retrograde transport machinery.

This system also allows the isolation of new mutant cells defective for intracellular retention of determinants contained in TMDs. Using a strategy identical to that previously described (22), we isolated new mutants unable to enter the intracellular localization of Ste2-P290D. None of the 14 mutants isolated was thermosensitive for growth. The retx mutant exhibited the most efficient mating (Fig. 8) and was not complemented with RET1, RET2, RET3, SEC21, or RER1. This suggests that it is mutated in a non-essential gene or that it represents a new, non-thermosensitive mutant allele of an already identified gene.

**DISCUSSION**

This work demonstrates that determinants in the transmembrane domains of type I membrane proteins can cause ER localization in yeast cells. These determinants are essentially composed of one or several hydrophilic residues in the membrane-spanning segment and are very similar to membrane determinants for ER localization identified previously in mammalian cells. Various features of mammalian membrane determinants (relative potency of various amino acid residues, effect of the length of the TMD) are also observed for yeast membrane determinants. This strongly suggests that a similar mechanism is involved in the recognition of these determinants in yeast and mammalian cells. However, one major difference should be noted; in contrast to what was observed in mammalian cells (9), ER retrieval signals in TMDs do not lead to non-lysosomal degradation in yeast cells. This suggests that these two events (ER localization and non-lysosomal degradation) can be distinguished mechanistically, and that some element of the targeting or degradation machinery is different in yeast cells.

Continuous retrieval to the ER is apparently crucial for the ER localization of proteins with membrane determinants. This is suggested by the fact that these proteins are localized in the ER but acquire sugar modifications typical of the early Golgi compartment. It is further confirmed by the fact that both coatomer and Rer1p are necessary for efficient ER localization of ER membrane determinants. Both coatomer and Rer1p were implicated previously in ER retrieval. Coatomer was shown to play an essential role in ER retrieval of dilsyne-tagged proteins from the Golgi to the ER (22). Rer1p was initially identified as a protein involved in the ER localization of Sec12p, a type II transmembrane protein involved in ER-to-Golgi transport (32–34). More recently it was shown that Rer1p participates in ER retrieval of Sec12p from the Golgi to the ER, a process critically dependent on the TMD of Sec12p (13). Though the targeting determinant in the Sec12p TMD remained elusive, it contains two hydrophilic residues (one asparagine and one glutamine) that are essential for ER retrieval of Sec12p (13). It is likely that similar features are recognized in the TMDs of type I membrane proteins studied here and of the type II membrane protein Sec12p. Our results also suggest that similar determinants can be recognized in the TMDs of polytopic membrane proteins, and that the same cellular machinery is involved, as suggested by the fact that Rer1p is essential for ER retrieval in both cases. Most polytopic proteins contain numerous charged residues within their predicted transmembrane spans, and evidently the positioning of the residue must be critical for its action as a sorting determinant. In the case of the KDEL receptor, it has been proposed that the polar residue critical for ER retrieval is exposed to the lipid bilayer, and thus in a position to be recognized by a membrane sorting machinery. It also remains to be determined whether a polar residue in any of the TMDs, or only in a subset of TMDs of a polytopic membrane protein can lead to ER retrieval.

These results stand in apparent contradiction with the recent report that the TMD of Ufe1p causes ER retention in a Rer1p-independent manner (14). Note, however, that our results do not exclude the possibility that membrane determinants also cause inefficient transport out of the ER. It is possible that some membrane determinants (e.g. Ufe1p) are more apt at causing true ER retention, while others act mostly by causing ER retrieval. The isolation of more mutants defective for ER localization of membrane determinants could lead to the identification of new components involved of the sorting machinery.

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