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Identification of a gene required for membrane protein retention in the early secretory pathway

(endoplasmic reticulum/Golgi apparatus/membrane recycling/Sec12 protein/*rer* mutations)

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ABSTRACT **The yeast *SEC12* gene product (Sec12p) is an integral membrane protein required for the protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus. Although this protein is almost exclusively localized in the ER, a significant fraction of Sec12p is modified by an enzyme that resides in the early compartment of the Golgi apparatus, suggesting that Sec12p is cycling between the ER and the early Golgi. We have taken a genetic approach to investigate the retention mechanism of Sec12p. Analysis of mutants that are defective in the retention of the Sec12-Mfα1 fusion protein in the early secretory compartments has identified a gene, *RER1*. A recessive mutation in *RER1* causes mislocalization of the authentic Sec12p as well as two different Sec12 fusion proteins to the late Golgi apparatus and even to the cell surface. However, the *rer1* mutant is not defective in the retention of an ER-resident soluble protein, BiP, suggesting that soluble and membrane proteins are retained in the ER by distinct mechanisms.**

In the secretory pathway, many proteins are exported to the cell surface by a default mechanism. Resident proteins of secretory organelles, such as the endoplasmic reticulum (ER) and the Golgi apparatus, have specific signals for retention in their residence. The best-known example of such signals is the C-terminal Lys-Asp-Glu-Leu (KDEL, mammals) and His-Asp-Glu-Leu (HDEL, yeast) sequences (1, 2) for soluble proteins in the ER, such as the immunoglobulin heavy chain binding protein (BiP). The receptor for the K(H)DEL sequence (Erd2p) has been identified in mammalian and yeast cells (3, 4). Recognition of this signal by the receptor has been suggested to take place in the early compartment of the Golgi complex and thus the retention of these ER proteins appears to be achieved by a recycling mechanism between the ER and the early Golgi (4–7). Recently, morphological evidence has been reported that ER membrane proteins, such as unassembled major histocompatibility complex class I molecules, also recycle between the ER and the cis-Golgi network (8). However, molecular mechanisms underlying such cycling events remain unclear.

Sec12p is an integral membrane protein required for formation of transport vesicles from the ER in yeast *Saccharomyces cerevisiae* (9, 10). Although subcellular fractionation (9) and immunofluorescence microscopy (this study; unpublished data) have demonstrated its steady-state localization in the ER, *N*-linked oligosaccharide chains on Sec12p are subject to modification by α-1,6 mannosyltransferase (11), which is believed to reside in the early compartment of the Golgi apparatus (12). These observations have led us to propose that Sec12p is also cycling between the ER and the early Golgi (9).

In this study, we have taken advantage of the powerful yeast genetics to try to prove the existence of such recycling mechanisms and, if they exist, to identify the cellular machinery involved. We have chosen the Sec12 protein as a tool for this approach. We constructed a fusion protein between Sec12p and a precursor of α-mating factor and introduced it in α-factor-deficient yeast cells. If this fusion protein is mislocalized to the late Golgi compartment, processing of the α-factor moiety would occur leading to the secretion of the mature pheromone. This trick has been used to screen for mutants that are defective in Sec12p retention. We describe in this paper the isolation of mutants, *rer1* and *rer2*, and show that *RER1* is in fact required for the normal localization of Sec12p.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions. The *S. cerevisiae* strains used in this study are listed in Table 1. Cells were usually grown in a rich medium containing 1% yeast extract, 2% polypeptone, and 2% glucose (YPD medium) or in Wickerham's minimal medium (15) containing 2% glucose (MVD medium) with appropriate supplements.

Plasmids. A 3.4-kb *Xho* I–*Xba* I fragment of pSEC1230 (9) containing the *SEC12* gene was subcloned into pBluescript SK$^+$ (Stratagene) and a yeast multicopy plasmid pSQ326 (16) to make pSHF2-1 and pSHY6-3, respectively. A *Hin*dIII site was introduced at the C-terminal (471st) codon of *SEC12* by site-directed mutagenesis of pSHF2-1 using an oligonucleotide, 5′-AAATAGATGAAGCTTGAGCTTG-3′. The resulting plasmid was designated pSHF3-2. The aspartate residue at position 470 changed to glutamate by this mutagenesis. The *SEC12-MFα1* fusion gene was constructed by inserting the *Pst* I (blunted by T4 polymerase) to *Xba* I fragment of pDJ100 (17) into the *Hin*dIII (blunted) and *Xba* I sites of pSHF3-2. The *SEC12-MFα1* gene was subcloned into pSQ326 to give pSHF9-4. To induce a frameshift mutation in the *SEC12* region of the fusion gene, pSHF9-4 was digested with *Sal* I, blunted with T4 DNA polymerase, and religated (pSHF9-4dSal). The *SEC12-SUC2* fusion gene was constructed by subcloning the *Xho* I–*Hin*dIII fragment of pSHF3-2 into the *Sal* I and *Hin*dIII sites of pSEY304 (18), and the resulting plasmid was designated YEpSEC12-SUC2. The junctions of the fusions were confirmed by nucleotide sequencing.

Halo Assay. Approximately 5×10^5 tester cells (*sst2*), which are supersensitive to α-factor, were spread onto MVD plates that contained 0.5% Casamino acids and appropriate supplements and were buffered at pH 3.5 (19). Cells to be tested for α-factor production were spotted on the plates with sterile toothpicks. The plates were incubated at 23°C for 13–16 hr and further incubated at 37°C for 24–30 hr. To examine halo production dependent on *SEC12-MFα1* fusion gene plasmid, host cells of the genotype *MATα bar1 mfα1 mfα2* were used.

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Abbreviations: ER, endoplasmic reticulum; BCP, bromocresol purple.

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Table 1. Yeast strains

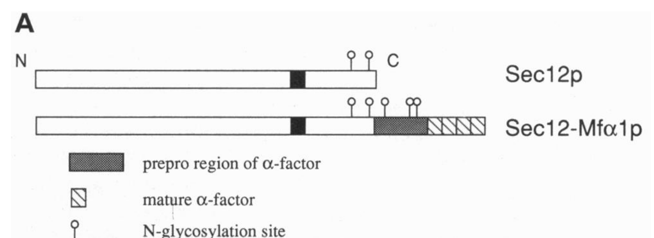
Strain	Genotype
SNY3	*MATa/MATα sec12::LEU2/sec12::LEU2 ura3/ura3 leu2/leu2 trp1/trp1 his/his* YEp[*SEC12 TRP1*]
SNY5	*MATa/MATα sec12::LEU2/sec12::LEU2 ura3/ura3 leu2/leu2 trp1/trp1 his/his* YEp[*SEC12-MFα1 TRP1*]
SNY9	*MATα ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNY16	*MATα suc2::LEU2 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNY22	*MATα pep4::LEU2 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNH6-1C	*MATα rer1-2 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNHA6-1C	*MATα rer1-2 pep4::LEU2 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNHS6-2A	*MATα rer1-2 suc2::LEU2 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNH23-10A	*MATα rer2-2 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNS218-3C	*MATa sec18-1 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
SNHS618-5A	*MATα rer1-2 sec18-1 ura3 lys2 ade2 trp1 his3 leu2 bar1::HIS3 mfα1::ADE2 mfα2::TRP1*
BC180	*MATa sst2-Δ2 ura3 leu2 his3 ade2*

All strains except BC180 were constructed in this study from either ANY102 (13) or YPH500 (14) by standard genetic methods. BC180 was a gift from W. Courchesne (University of Nevada).

Mutant Isolation and Screening. SNY9 cells containing pSHF9-4 were grown to 1×10^8 per ml in MVD medium containing 0.5% Casamino acids at 30°C. Cells (1×10^8) were mutagenized with ethyl methanesulfonate or nitrous acid as described (20). The viability of yeast cells after mutagenesis was 42% for ethyl methanesulfonate and 12–16% for nitrous acid treatment. After 17 hr of incubation at 23°C in MVD medium containing 0.5% Casamino acids, the mutagenized cells were inoculated on 25 MVD plates containing 0.5% Casamino acids (100–200 cells per plate). After 4 days at 23°C, colonies were replicated onto halo assay plates, which were incubated at 23°C for 16 hr and further incubated at 37°C for 2 days. Colonies that produced a halo on the replica plates were picked up and spotted on fresh halo assay plates. Cells that reproducibly showed the $Halo^+$ phenotype were streaked on MVD plates containing 1 mg of 5-fluoroorotic acid per ml to toss the fusion plasmid. Mutant clones that showed the $Halo^+$ phenotype when retransformed with pSHF9-4 were saved, back-crossed with the parent strain at least three times, and subjected to further characterization.

RESULTS AND DISCUSSION

Isolation of Mutants that Mislocalize Sec12-Mfα1 Protein to the Late Golgi Compartments. Sec12p is strictly localized in the ER. To confirm our previous conclusion by subcellular fractionation (9), we performed indirect immunofluorescence experiments using anti-Sec12p antibodies. Fig. 1*B* shows a typical picture of the staining of Sec12p in Sec12p-overproducing cells. The staining of nuclear envelope and tubular extensions in the cytoplasm is typical of yeast ER proteins. In fact, double staining with anti-Sec12p and anti-BiP antibodies showed the colocalization of the two proteins (data not shown). There was little, if any, indication of the Golgi staining. This ER localization was not due to the overexpression of Sec12p, since a similar but more restricted staining of the ER membrane was observed in the wild-type cells (not shown). On the other hand, a significant portion of the Sec12p molecules is subject to $\alpha 1 \rightarrow 6$ mannosyl modification, which is thought to occur in a very early compartment of the Golgi apparatus (11). Our hypothesis that Sec12p recycles between the ER and the Golgi is based on these observations. The Golgi modification on Sec12p is again not due to the overproduction of the protein because it is also observed in the wild type (unpublished data). As will be shown later, Sec12p never acquires $\alpha 1 \rightarrow 3$ mannosyl linkages, which are added in a later compartment of the Golgi. This suggests that Sec12p travels only to the early Golgi during cycling.



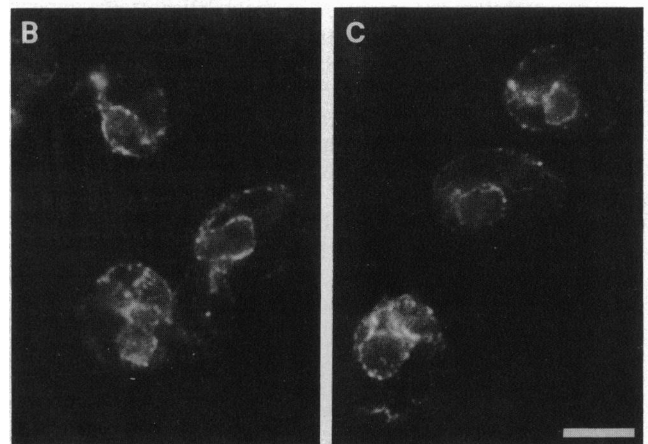


FIG. 1. Construction of the *SEC12-MFα1* fusion gene. (*A*) Schematic representation of the fusion. (*b* and *c*) Analysis of subcellular localization of Sec12p and Sec12-Mfα1p by immunofluorescence microscopy. The *sec12* null mutant cells with a multicopy plasmid containing the *SEC12* gene (SNY3, *B*) or the *SEC12-MFα1* gene (SNY5, *C*) were grown in the YPD medium at 30°C and prepared for immunofluorescence microscopy with the anti-Sec12p antibody. (Bar = 5 μm.)

We reasoned that a defect in the recycling mechanism of Sec12p would cause mislocalization of the protein to the late Golgi and further to the cell surface or vacuole by default. If so, we should be able to isolate mutants, which will be very useful to verify our hypothesis. As a tool for mutant screening, we constructed a *SEC12-MFα1* fusion gene by hooking *MFα1*, a structural gene for α-mating factor (21), in frame at the end of the *SEC12* open reading frame (Fig. 1*A*). α-Factor is a secretory protein of yeast, and its modifications during the transport through the secretory pathway have been most extensively investigated. The molecules of pro-α-factor are subject to various modifications during the transit through the Golgi apparatus. α1 → 6 mannose residues are added to *N*-linked oligosaccharide chains in an early Golgi compartment as mentioned above. Addition of α1 → 2- and α1 → 3-linked mannose to the α1 → 6 mannosylated form and Kex2p-dependent proteolytic processing take place successively at distinct late Golgi compartments (12, 22, 23).

The *SEC12-MFα1* fusion gene complemented the lethality of the *sec12* null mutation even on a single-copy plasmid, indicating that it retains the essential function of *SEC12*. Immunofluorescence microscopy showed that the fusion protein is also localized in the ER (Fig. 1*C*). Furthermore, the Sec12-Mfα1 protein acquired α1 → 6 mannose but not α1 → 3 mannose linkages (not shown), indicating that this fusion protein behaves completely like the authentic Sec12p. If this chimeric protein is mislocalized beyond the early Golgi, the Kex2 protease in the late Golgi will act on the Mfα1 moiety, leading to the production of the mature α-factor. The secretion of α-factor can be detected by a sensitive halo assay (24). In the wild-type cells, the *SEC12-MFα1* fusion did not yield a halo (see Fig. 2), confirming that the fusion protein is restricted only to the ER and the early Golgi.

By screening 14,000 colonies of mutagenized yeast cells, we isolated four mutant clones that showed plasmid-dependent secretion of α-factor. They all contained a single recessive mutation, which segregated 2:2 when crossed with the wild type and defined two complementation groups by mutual crosses. Expecting that they were defective in the retention in the ER or in the return to the ER from the early Golgi of Sec12p, we named these mutants *rer1* and *rer2*. Two different methods of mutagenesis gave rise to one allele each of *rer1* and *rer2*. As shown in Fig. 2, *rer1* produced a larger halo than *rer2*, but there was no appreciable difference between the two alleles. Fig. 2 also shows that a frameshift mutation in the *SEC12* portion abolished the Halo$^+$ phenotype, ruling out the possibility that the halo was due to the truncated transcription or translation of the *MFα1* moiety. α-Factor production by the *rer* mutants was *KEX2* dependent (not shown), indicating that at least a fraction of the fusion protein had reached the Kex2p-residing compartment.

The *rer1* Mutant Is Defective in the Retention of Wild-Type Sec12p. Characterization of the *rer1* mutant was pursued further. First, processing of the *bona fide* Sec12 protein was

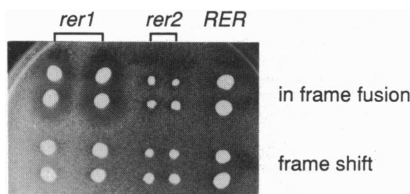


FIG. 2. Halo assay of the isolated *rer* mutants. Wild-type (*RER*) and *rer* mutant cells harboring a multicopy plasmid containing the *SEC12-MFα1* gene (pSHF9-4, upper row) or the frameshift mutant of the fusion gene (pSHF9-4dSal, lower row) were examined for α-factor production by halo assay. All *rer* mutants were derived from SNY9. Strains shown are (from left to right): H1 (*rer1-1*), H6 (*rer1-2*), H21 (*rer2-1*), H23 (*rer2-2*), and SNY9 (*RER*).

examined by a pulse–chase experiment (Fig. 3). In the wild-type cells, Sec12p showed a slow increase in the molecular mass up to 69 kDa, which was mostly due to the progressive modification on *O*-linked sugar chains (9). As mentioned above, a significant fraction of Sec12p acquired α1 → 6 mannose residues on its *N*-linked sugar chains during the chase period, whereas the addition of α1 → 3 mannosyl linkages was not observed (Fig. 3*a*, left). In the *rer1* mutant, however, the change in the mobility was much more dramatic. A smear species that extends above 70 kDa was seen after a 2-hr chase (Fig. 3*a*, right). The heterogeneity of modification was already obvious even at a 1-hr chase. As the highly modified species collapsed to a narrow band by endoglycosidase H treatment (see Fig. 3*b*, upper), the unusually extensive elongation must have taken place on the *N*-linked oligosaccharides. This smear species was precipitable with anti-α1 → 3 mannose antibody (Fig. 3*a*, right), indicating that it was produced by the modification of Sec12p by the late Golgi enzyme(s). This strongly suggests that the *rer1* mutant has a defect in proper localization of Sec12p. However, these observations could also be explained if the *rer1* mutation caused accumulation or mislocalization of the late Golgi enzymes in the ER. To test this possibility, we constructed a *sec18 rer1* double mutant. If Sec12p is indeed mislocalized in *rer1*, the block of ER-to-Golgi transport by *sec18* should prevent the Golgi-specific modifications of Sec12p. Fig. 3*b* (lower) shows the result of a pulse–chase experiment of *sec18* alone and *sec18 rer1* double mutant cells at 37°C, the restrictive temperature of *sec18*. Under this condition, the formation of both α1 → 6 and α1 → 3 species was almost completely inhibited. Therefore, even in the *rer1* mutant, Sec12p has to reach the Golgi apparatus to encounter those Golgi enzymes. The integrity of the Golgi apparatus seemed to be normally maintained in this mutant. Processing of a vacuolar protein, carboxypeptidase Y, occurred in the *rer1* mutant as efficiently as in the wild-type cells (data not shown). It is again unlikely that the *rer1* mutation retards ER-to-Golgi transport leading to the accumulation of Golgi enzymes in the ER. Furthermore, immunofluorescence observation with the anti-Kex2p antibody revealed normal Golgi staining in *rer1* that was indistinguishable from the wild type (not shown). Thus, the α1 → 3 mannosyl modification observed in *rer1* is a demonstration that the Sec12 protein was mistransported to the late compartments of the Golgi apparatus.

The mislocalization phenotype of *rer1* was further tested for another chimeric protein, Sec12-Suc2p, which was constructed by fusing *SUC2*, a gene encoding invertase, to *SEC12* (Fig. 4*a*). This fusion protein was shown to preserve Sec12p and invertase activities. If the invertase activity is secreted to the cell surface, hydrolysis of sucrose can be monitored on an indicator plate containing BCP. As shown in Fig. 4*b*, when the invertase-secreting cells (*SUC2 RER1*) grew on the BCP/sucrose plate, the purple color turned yellow (bottom). In the *RER1 suc2* cells, the Sec12-Suc2 protein was retained intracellularly and thus no invertase activity was detected on the cell surface (see upper left). In contrast, the *rer1 suc2* mutant cells harboring the *SEC12-SUC2* plasmid clearly exhibited invertase activity on this plate (lower right). This was not due to the secretion of invertase that was formed by degradation of the fusion protein, because the cell surface activity of invertase was membrane bound. As shown in Table 2, 14% of the total invertase activity was detected extracellularly, but its major part remained unreleased by spheroplasting. The invertase activity of the normal *SUC2* product was mostly liberated into the periplasm (not shown). In this experiment, only a very low activity of the cytosolic marker, glucose-6-phosphate dehydrogenase, was released by spheroplasting, indicating that cell lysis was negligible. The possibility that the membrane-bound invertase activity was due to lysed cell

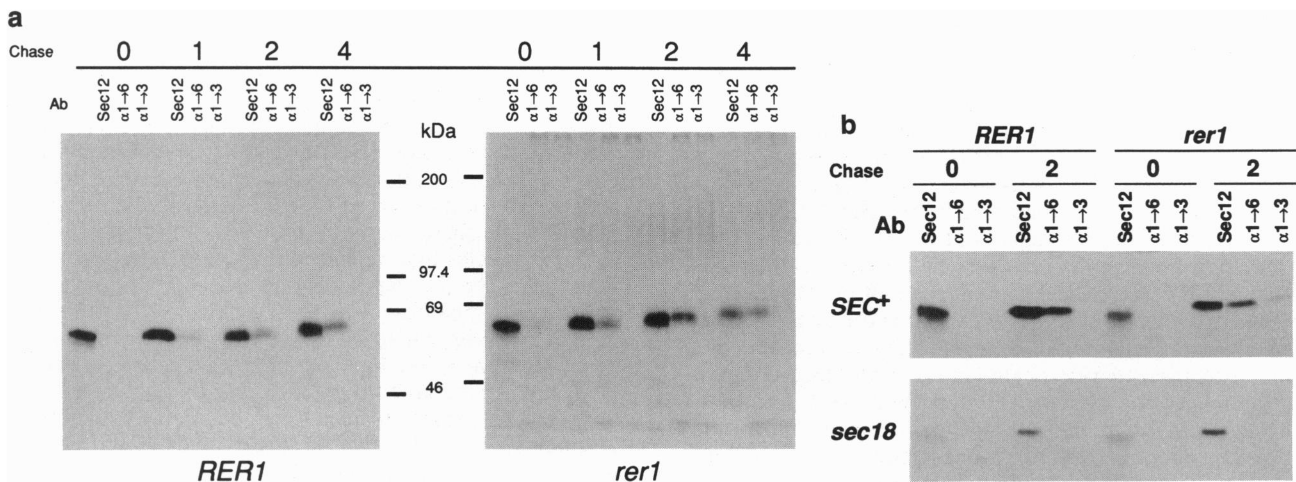


FIG. 3. Pulse–chase analysis of Sec12p in the *rer1* mutant. (*a*) Wild-type (SNY22, left) and the *rer1* mutant (SNHA6-1C, right) harboring a multicopy plasmid containing the *SEC12* gene (pSHY6-3) were labeled with Tran^{35}S-label for 10 min at 30°C and chased for times indicated (in hours) above the lane. Sec12p was first immunoprecipitated with anti-Sec12p antiserum and the immunoprecipitated materials were subjected to the second round immunoprecipitation with antibodies (Ab) against Sec12p, $\alpha 1 \rightarrow 6$ mannose, and $\alpha 1 \rightarrow 3$ mannose linkages. (*b*) Metabolic labeling and immunoprecipitation were performed as described above, except that the *sec18* mutant cells (SNS218-3C, *sec18 RER1*; SNHS618-5A, *sec18 rer1*) were labeled and chased at 37°C to impose the ER-to-Golgi transport block. Immunoprecipitated materials were treated with endoglycosidase H and subjected to SDS/PAGE and fluorography.

fragments produced during culture is also ruled out because the activity of an ER membrane enzyme, NADPH-cytochrome *c* reductase, was almost completely latent in the *rer1* cell pellet (data not shown). These results indicate that the Sec12-Suc2 fusion protein was also mislocalized to the cell surface. The reason why only a small amount of Sec12-Suc2p was exported may be due to the leakiness of the *rer1* mutation. Alternatively, a larger portion of the fusion protein could be transported to the vacuole by default (27) and degraded there.

Taking all of these pieces of evidence together, we conclude that *rer1* has a defect in the retention of the Sec12 protein in the early compartments of the secretory pathway.

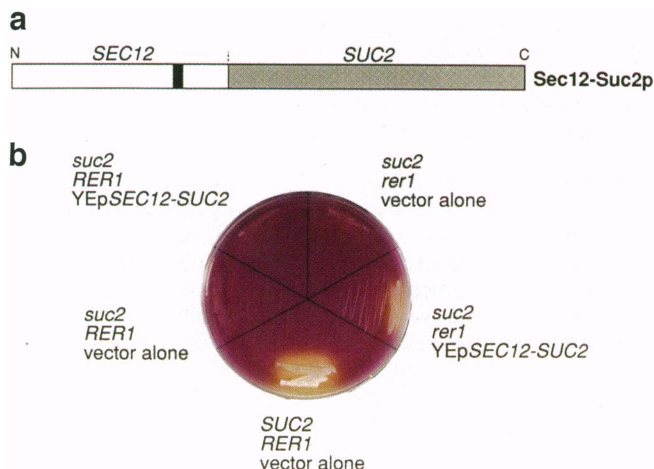


FIG. 4. The *rer1* mutant mislocalizes Sec12-invertase fusion protein to the cell surface. (*a*) Schematic representation of the Sec12-Suc2 fusion protein. Almost all coding frame of mature invertase (from the third codon, hatched box) was fused in frame to the C terminus of Sec12p (open box, filled region indicates the membrane span). (*b*) Detection of the extracellular invertase activity on the bromocresol purple (BCP)/sucrose plate. The BCP/sucrose plate contained 0.67% yeast nitrogen base, 0.5% Casamino acids, 2% sucrose, 0.0032% BCP, and 2% agar. Cells were streaked on the BCP/sucrose plate and incubated at 30°C for 2 days. Strains shown are SNY9 (*SUC2 RER1*), SNY16 (*suc2 RER1*), and SNHS6-2A (*suc2 rer1*). YEpSEC12-SUC2 is a multicopy plasmid containing the *SEC12-SUC2* fusion gene.

This supports the view that a cellular mechanism exists to restrict Sec12p to the ER and the early Golgi. The defect of *rer1* could be due to a lesion in the retrograde movement from the early Golgi to the ER. It should be pointed out here that the transport from the ER to the early Golgi still seems to be rate limiting in the *rer1* cells. As shown in Fig. 3*a*, acquisition of the $\alpha 1 \rightarrow 6$ mannosyl linkages requires a very long time even in *rer1*. This might indicate that there is another mechanism to restrict Sec12p, perhaps at the step of exit from the ER. Although we tried to incorporate a temperature-sensitive screening step in mutant isolation, the obtained *rer1* mutants showed a constitutive defect. Determination of whether *RER1* fulfills an essential function for cell growth awaits its cloning and gene disruption experiments.

Retention of BiP Is Normal in the *rer1* Mutant. It has been shown that ER soluble proteins such as BiP (the *KAR2* gene product in yeast) are retained in the ER by the K(H)DEL recognition system (2). The *erd* mutants, which are defective in ER retention of BiP, secrete the protein into the culture medium (28, 29). We tested whether the *rer* mutants display such an Erd$^-$ phenotype. As shown in Fig. 5, the amount of BiP detected in the culture medium of *rer1* was as little as that of the wild type. In this mutant, the cellular level of BiP was indistinguishable from that of the wild-type cells (not shown). This indicates that BiP is normally retained in the *rer1* mutant.

Table 2. Extracellular invertase activity of *rer1* is membrane bound

	% total			
	rer1		*RER1*	
Fraction	Invertase	G6PDH	Invertase	G6PDH
Periplasmic	4	2	1	1
Spheroplast surface	10	3	3	3
Intracellular	86	95	96	96

SNHS6-2A (*rer1*) and SNY16 (*RER1*) cells harboring the YEpSEC12-SUC2 plasmid were harvested at an early logarithmic phase and treated with Zymolyase. Periplasmic and spheroplast fractions were separated by centrifugation and assayed for invertase (25) and glucose-6-phosphate dehydrogenase (G6PDH) (26) activities. Activities on the spheroplast surface and the whole activities associated with the spheroplasts were assayed in the absence and presence of 0.1% Triton X-100, respectively. "Intracellular" represents whole spheroplasts minus spheroplast surface.

Recently, a retention signal for ER membrane proteins has been identified in animal cells. Two lysine residues at the third and the fourth or fifth positions from the C terminus function as the ER retention signal of the adenovirus E19 protein (30). The *WBP1* gene product, a yeast homologue of the 48-kDa subunit of oligosaccharyltransferase, has been reported to have this type of retention signal (31). In contrast, yeast Sec20 and Sed4 proteins are examples of membrane proteins that harbor the HDEL motif (32, 33). Sec12p has neither HDEL nor Lys-(Xaa)-Lys-Xaa-Xaa motif yet localizes in the ER, implying the presence of a signal for ER retention. Pelham and his coworkers have reported that a set of *sec* mutants (*sec17, sec18, sec20*, and *sec22*) secreted BiP at the permissive temperature (29). However, these mutant cells did not produce a halo in our assay when transformed with the *SEC12-MFα1* fusion plasmid (not shown), indicating that these mutants are normal in the retention of Sec12p. These observations as well as the above finding that only Sec12p but not BiP is mislocalized in *rer1* implicate multiple sets of cellular machinery functioning in the protein sorting between ER and Golgi.

Do multiple pathways of the ER–Golgi recycling exist then? It may be too early to speculate at this point but a very intriguing finding has been recently reported by Pelham's group (33, 34). They isolated multicopy suppressor genes that overcome the lethality of *ERD2* disruption. Among five genes obtained, two were previously unknown but the others were known genes: *SEC12, DPM1* encoding dolichol-P-mannose synthase, and *SED4*, a gene that was already found in the yeast chromosome III with high similarity to *SEC12* (open reading frame YCR67c) (35). Why do these genes suppress the deletion of the HDEL receptor? Provided that there are two distinct routes of backward movement from the Golgi—i.e., *ERD* pathway and *RER* pathway—they might well interact with each other. The knockout of the *ERD* pathway is lethal but one may be able to remedy the defect by stimulating the *RER* pathway by increasing the passenger—e.g., Sec12p. We do not know at present whether the other suppressor genes of *Δerd2* are under control of the *RER1* gene. As mentioned above, Sed4p is a membrane protein with the C-terminal HDEL sequence. Its interaction with the Erd2 protein remains to be examined. Of course, many more complex, direct or indirect, models are all possible. Cloning and analysis of the *RER1* gene will be necessary to exploit these possibilities. We have already mapped the *rer1* locus in the chromosome III, thus excluding the possibility that *rer1* is allelic to *erd1* or *erd2*. Exact mapping is necessary, but the *rer1* locus is apparently distinct from those for genes of immediate interest, such as *SED4* and *ERS1* (suppressor of *erd1*; ref. 36). In regard to the relationship between *erd* and *rer* mutations, it may also be of interest that *rer2* shows elevated secretion of BiP. We have observed aberrant protein glycosylation and high expression of BiP in the *rer2* mutant (unpublished data). One easy explanation is that the secretion of BiP by *rer2* is due to its overproduction, but apparently we need more work to understand the function of *RER2*.

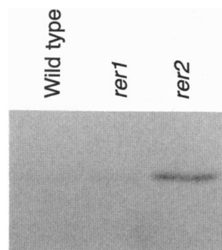


FIG. 5. Analysis of the missecretion of BiP. Wild-type (SNY9) and *rer* mutant cells (*rer1*:SNH6-1C and *rer2*:SNH23-10A) were grown in YPD medium overnight to the early logarithmic phase. Proteins from the culture medium (equivalent to 2.5×10^6 cells) were analyzed by immunoblotting with the antibody against yeast BiP.

The genetic approach we have taken will provide powerful tools to unveil components of sorting machinery involving dynamic vesicular traffic. Analysis of the *RER* genes will give us a clue to understanding molecular mechanisms underlying membrane recycling in the early secretory pathway, especially the retrograde movement from the Golgi to the ER, which remains largely unexploited.

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