Degradation of Endoplasmic Reticulum (ER) Quality Control Substrates Requires Transport between the ER and Golgi

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Endoplasmic reticulum (ER) quality control (ERQC) components retain and degrade misfolded proteins, and our results have found that the degradation of the soluble ERQC substrates CPY* and PrA* but not membrane spanning ERQC substrates requires transport between the ER and Golgi. Stabilization of these misfolded soluble proteins was seen in cells lacking Erv29p, a probable Golgi localized protein that cycles through the ER by means of a di-lysine ER retrieval motif (KKKY). Cells lacking Erv29p also displayed severely retarded ER exit kinetics for a subset of correctly folded proteins. We suggest that Erv29p is likely involved in cargo loading of a subset of proteins, including soluble misfolded proteins, into vesicles for ER exit. The stabilization of soluble ERQC substrates in both erv29Δ cells and sec mutants blocked in either ER exit (sec12Δ) or vesicle delivery to the Golgi (sec18Δ) suggests that ER-Golgi transport is required for ERQC and reveals a new aspect of the degradative mechanism.

Proteins entering the secretory pathway are first translocated into the endoplasmic reticulum (ER)† where they undergo folding and maturation involving either N- or O-glycosylation, oligomerization, disulfide bond formation, or subunit assembly. Misfolded proteins are thought to be selectively retained in the ER by the quality control (ERQC) system and if unable to refold correctly, the misfolded proteins are retrotranslocated to the cytosol via the translocon to be ubiquinated and degraded by the proteasome (1–5). This ER-associated degradation of misfolded proteins is common to both higher eukaryotic cells and the yeast Saccharomyces cerevisiae in which known components of the ERQC include DER1, HRD1/DER3, and HRD3 (6–8) as well as the ubiquitin-conjugating enzymes UBC6 and UBC7 (5). Known soluble substrates of the ERQC in yeast include CPY* and PrA*, mutant forms of the yeast vacuolar enzymes carboxypeptidase Y and proteinase A, respectively (9). Examples of membrane spanning ERQC substrates in yeast include Vphlp*, a misfolded subunit of the yeast vacuolar H+-ATPase, and Sec61-2p, a mutant form of the major translocon component (10).

Extensive protein misfolding occurs in cells treated with either tunicamycin, which prevents N-linked glycosylation, or dithiothreitol (DTT), which prevents disulfide bond formation, and the resulting accumulation of misfolded proteins in the ER activates the unfolded protein response (UPR) (11–13) to transcriptionally up-regulate a group of genes to assist in relieving the ER stress. UPR activation is initiated by Ire1p, a transmembrane kinase/nuclease (14, 15) that performs the unorthodox splicing of HAC1 mRNA (16–19). The resulting active transcription factor, Hac1p (18, 20), binds to the UPR element (UPRE) found in the promoter region of target genes to induce their transcription (17, 18, 21).

Microarray data on yeast cells stressed with tunicamycin and DTT have shown that the UPR up-regulates a wide range of genes whose encoded proteins act upon different aspects of the ER to relieve the stress induced by the misfolded proteins (12, 13). Not surprisingly, known components of the ER degradative pathway are up-regulated including DER1, HRD1/DER3, HRD3, and UBC7. The transcription of genes encoding ER molecular chaperones including KAR2 was also induced presumably to assist in refolding the misfolded proteins. Another category of genes up-regulated by the UPR is that involved in transport between the ER and Golgi. This category included genes involved in vesicle budding from the ER (SEC12, SEC13, SEC16, and SEC24), retrograde transport from the Golgi to the ER (ERD2, RER2, RET2, and SEC26), and a gene thought to be involved in cargo selection for ER exit, ERV25 (13).

Properly folded secretory proteins are packaged and exported from the ER and transported by COPII-coated vesicles to the Golgi to be further modified and sent to their final destination (22–25). A family of ERV (ER vesicle) proteins including Erv14p, Erv25p, Erv41p, and Erv46p have been isolated from the ER-derived COPII vesicles and are thought to function in vesicle formation, cargo loading, and/or membrane fusion (26–28). An additional family of transmembrane proteins associated with COPI and COPII vesicles, known as p24 proteins (Emp24p, Erv25p, and Erp1–Erp6), is also proposed to be involved in vesicle formation, cargo protein sorting, and regulation of vesicular transport and is required for forward transport of a select subset of secretory proteins (29).

This work searched for genes encoding additional components of the ERQC responsible for the degradation of misfolded proteins in the ER of S. cerevisiae. A number of genes up-regulated by the UPR with unknown function were screened for stabilization of an ERQC substrate from which YGR284c/ERV29 was found to be involved in the degradation of soluble ERQC substrates. Here we characterize the function and localization of Erv29p. Stabilization of soluble ERQC substrates was observed in cells that had transport blocked between

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† The abbreviations used are: ER, endoplasmic reticulum; ALP, alkaline phosphatase; CPY, carboxypeptidase Y; CPY*, misfolded form of CPY; DTT, dithiothreitol; ERQC, ER quality control; HA, hemagglutinin; PrA, proteinase A; PrA*, misfolded form of PrA; UPR, unfolded protein response; UPRE, unfolded protein response element; COPI, coat protein complex type I; COPII, coat protein complex type II; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis.

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the ER and Golgi, indicating that ER-Golgi transport is necessary for soluble ERQC substrate degradation.

**EXPERIMENTAL PROCEDURES**

**Materials**—The restriction enzymes were purchased from New England Biolabs. The oligonucleotides were purchased from IDT. The chemicals were purchased from either Fisher or Sigma. The yeast strains were purchased from Research Genetics.

**Strains, Plasmids, Media, and Microbiological Techniques**—The media were prepared as described by Hill and Stevens (30). The yeast strains used in this study are listed in Table I. To introduce the following alleles (erv41::KAN, erv25::KAN, erv29::KAN, erv46::KAN, emp24::KAN), and erv42::::KAN into new strains, DNA corresponding to the disrupted alleles available from the *S. cerevisiae* Genome Deletion Project (distributed by Research Genetics) using oligonucleotides specific for each locus. The amplified DNA product was then transformed into the relevant strains with selection on YEPD pH 5.0 medium containing 200 μg/ml G418 (Calbiochem). The structure of each disruption was confirmed by PCR analysis of genomic DNA utilizing oligonucleotides flanking each disrupted locus.

**ER Quality Control Degradation Requires ER-Golgi Transport**

**HA epitope tagging of ERV29** was performed by first amplifying a 1.9-kilobase ERV29 fragment from genomic DNA isolated from SEY6211, which was then inserted into pCR 2.1-TOPO to create pAC506. A 2.0-kilobase NotI-BamHI ERV29 fragment from pAC506 was ligated into NotI-BamHI-digested pRS313 to create pAC505. pAC505 was cut with BamHI, the ends blunted by Klenow enzyme, and religated to destroy the BamHI site within the polylinker and create pAC520. A BamHI site was introduced 136 base pairs from the start codon of ERV29 in pAC520 using a QuickChange XL Mutagenesis kit (Stratagene) to create pAC529. A 120-base pair BglII fragment containing three influenza virus hemagglutinin epitopes from BJ7122 (kindly provided by Dr. Elizabeth Jones) was inserted into BamHI-digested pAC529 to create pAC530. The ERV29-HA construct was confirmed by Western blotting with anti-HA antibodies and the subsequent complementation of the CPY* stabilization phenotype observed in erv29Δ cells.

**ERQC Protein Degradation Requires ER-Golgi Transport**—The whole cell protein extracts were prepared as described (30). The secondary antibodies were purchased from Bio-Rad. The immunoblots were detected using chemifluorescent or chemiluminescent detection (Amersham Pharmacia Biotech). The polyclonal antibodies were generated by HTI Bio-Products Inc. The monoclonal antibodies were purchased from Molecular Probes. Immunofluorescence microscopy was performed as described (30). Sodium carbonate fractionation was performed as described (35).

**Radialabeling and Immunoprecipitation**—Radialabeling and immunoprecipitation were performed as described (35). Samples were resolved by SDS-PAGE, and the gels were fixed, dried, and exposed either to a phosphor cassette (Molecular Dynamics) or to x-ray film. Quantitation of gels was as described (35).

**RESULTS**

Our interest in the ER quality control led us to investigate genes that are transcriptionally up-regulated upon ER stress for their potential involvement in the degradation of ERQC substrates. We examined recent microarray data of ER-stressed *S. cerevisiae* and identified a number of uncharacterized candidate genes (Table II) based on up-regulation following ER stress induced by both expression of an ERQC substrate (12) and the compounds DTT and tunicamycin, which inhibit protein folding in the ER (13). We obtained 13 strains individually disrupted for these genes and introduced a HA-tagged allele of CPY*, a well characterized ERQC substrate (8, 9). The half-life of CPY*-HA in parent and mutant strains was determined by radialabeling and immunoprecipitation. As an internal control we also included a strain that was disrupted in *HRD1/DER3* as Hrd1p is required for efficient degradation of CPY* (6). Table II indicates that CPY*-HA was stabilized in *hrd1Δ* cells as expected and was also stabilized in the strain disrupted in the YGR284c open reading frame.
Stabilization of Soluble ERQC Substrates in ygr284cΔ

Cells—The YGR284c open reading frame was disrupted in cells carrying an integrated pre1-1 (CPY*) allele and examined for any stabilizing effects. The rate of CPY* degradation in KHY270 (ygr284cΔ) cells was slowed considerably compared with the parent strain KHY163 (Δygr284c) (Fig. 1, A and C). The effect of a YGR284c disruption was then tested on other ERQC substrates, and it was found that PrA* (9) was also stabilized (t1/2 = 18 min versus 52 min) but Sec61-2p (10) and Vph1p* (35) were not (Fig. 1, B, D–F). These results suggested that the YGR284c-encoded protein (Ygr284p) is involved in the degradation of soluble ERQC substrates (CPY* and PrA*) but not membrane-spanning substrates (Sec61-2p and Vph1p*).

Ygr284p Is an ER Integral Membrane Protein Up-regulated by ER Stress—The sequence of the YGR284c open reading frame predicts a 310-amino acid protein, Ygr284p, with a predicted molecular mass of 35 kDa and hydrophobic stretches sufficient to span a membrane up to six times (YPD database Proteome, Inc.). Data base searches revealed similarities between Ygr284p and the SURF4 family of proteins with which it shares ~35% identity. The SURF4 family comprises Surfeit locus protein 4 from mouse and human and homologues in Drosophila melanogaster, Schizosaccharomyces pombe, and Caenorhabditis elegans; however, no known functions have been assigned for these proteins (Fig. 2A). The COOH terminus sequence of Ygr284p is KKKIY and matches the consensus di-lysine based ER retrieval motif KKXX for membrane proteins (36). This retrieval motif is likely functional because it is conserved among members of the SURF4 family (Fig. 2B, C). The COOH-terminal exposure of the KKXX motif, renders the protein nonfunctional as judged by a failure to restore CPY* degradation in Ygr284Δ cells (data not shown).

The unfolded protein response (UPR) up-regulates the expression of a subset of genes in response to stress in the ER, and YGR284c has been shown to be transcriptionally up-regulated under such conditions (12, 13). The UPR is mediated through UPREs found in the promoter regions of UPR genes to which the transcription factor Hac1p binds during ER stress (16, 18). We examined the promoter region of YGR284c and identified a likely UPRE at positions −106 to −85 (relative to a translational start site of +1). Fig. 2B shows a comparison of the potential UPREs found in the YGR284c locus with UPREs from genes known to be up-regulated by the UPR including KAR2, PDI1, LHS1, SCJ1, KFB2, and EUG1 (16).

The UPR can be induced by tunicamycin or DTT, compounds that induce misfolding of proteins within the ER by preventing N-linked glycosylation or that reduce disulfide bonds, respectively (37, 38). The cells in which the UPR had been induced by the addition of tunicamycin or DTT were examined for any effects on the level of Ygr284p. KHY271 (ygr284Δ) cells expressing pAC530 (Ygr284p-HA) were treated with either 10 μg/ml tunicamycin or 2.5 mM DTT for 30°C, and cellular extracts were prepared. Equal amounts of protein were immunoblotted and probed with anti-HA antibodies (Fig. 2C). The 3.5–4.7-fold increase in Erv29p observed when cells were stressed with tunicamycin or DTT correlated well with the 2.8–3.5-fold transcriptional up-regulation of Erv29p detected by microarray analysis (13).

Ygr284p Is an Integral Membrane Protein of the Early Secretory Pathway—The sequence of YGR284c predicts a protein with 5–6 potential membrane spanning domains. To confirm that Ygr284p was an integral membrane protein, we performed sodium carbonate (pH 11.5) fractionation on cell lysates, a procedure that solubilizes all but integral membrane proteins (39). KHY271 cells carrying pAC530 (Ygr284p-HA) were lysed, treated with sodium carbonate (100 mM), and separated into membrane pellet and soluble fractions. Equivalent amounts of protein were immunoblotted and probed with anti-HA antibodies. The fractionation profile for Ygr284p was the same as for the integral membrane protein, Dpm1p (Fig. 3A), and the con-
verse of the soluble protein phosphoglycerol kinase (PGK).

The subcellular localization of Ygr284p-HA was determined by indirect immunofluorescence of Ygr284p-HA (pAC530) in KHY271 (ygr284A) cells. The presence of a consensus di-lysine based ER retrieval signal at the COOH terminus of Ygr284p-HA predicted an ER staining pattern as seen with the ER resident protein, Eug1p (Ref. 40 and Fig. 3B). However, the staining pattern of Ygr284p-HA was clearly distinct from the typical ER staining pattern of Eug1p and was most similar to that of proteins localized to the Golgi complex (Fig. 3B). Schröder et al. (41) reported a similar staining pattern for Emp47p, which, although possessing a functional di-lysine motif, was localized to the Golgi by immunofluorescence and fractionation (41). The cells that expressed untagged Ygr284p showed no specific staining with anti-HA antibodies (Fig. 3B).

Ygr284p/Erv29p Is Involved in ER-Golgi Trafficking—The assignment of YOR284c as ERV29 by the Saccharomyces Genome Database and subsequent publication informed us that Ygr284p/Erv29p could be isolated in ER-derived COP1 vesicles and suggested a role for Erv29p in ER-Golgi transport (27). Erv14p, Erv25p, and recently Erv41p and Erv46p have all been identified in ER-derived COP1 vesicles, and their individual absences has varying effects on the trafficking of correctly folded proteins from the ER to the Golgi (26–28). We investigated the role of Erv29p in ER-Golgi transport by examining the forward transport of correctly folded soluble and membrane spanning secretory proteins in wild type cells and those lacking Erv29p. CPY is a useful indicator of trafficking through the secretory pathway because its molecular mass varies as it transits to the vacuole; the ER p1 precursor form (67 kDa) is further glycosylated within the Golgi complex (p2, 69 kDa) and then processed to the mature form (m, 61 kDa) in the vacuole (42). Fig. 4A shows that the absence of Erv29p strongly delays the trafficking of CPY to the vacuole. There is a very prolonged presence of the p1 ER form throughout most of the 60-min chase period, whereas in wild type cells both the Golgi-modified p2 and the mature vacuolar form are visible even in the pulse lane. The delayed trafficking of CPY in erv29Δ cells showed no Golgi-modified p2 form, which could be due to the fact that (i) trans-Golgi trafficking was rapid compared with the ER exit rate, (ii) erv29Δ cells have a disturbed Golgi function that would inhibit the Golgi localized mannosyltransferases responsible for converting p1 CPY to p2, or (iii) CPY is delivered to the vacuole by alternative means in erv29Δ cells. To distinguish between these possibilities Pep4 was disrupted in erv29Δ cells to abolish the proteolytic maturation of CPY in the vacuole so that any p2 form would then accumulate in the vacuole instead of being processed to the mature form. KHY278 (erv29Δ pep4Δ) cells slowly accumulate the p2 form (data not shown), suggesting that CPY does transit to the Golgi but that ER export is delayed in erv29Δ cells.

The lack of Erv29p had a similar severe effect on the ER to vacuole trafficking of PrA. The appearance of the mature vacuolar form of PrA was significantly retarded in erv29Δ cells relative to that in wild type cells (Fig. 4B). However, not all proteins trafficking from the ER are delayed in erv29Δ cells because the secreted soluble protein invertase with its extensive Golgi-derived glycosylation pattern appears indistinguishable in both wild type and erv29Δ cells (Fig. 4D). In addition, the transit time of the membrane spanning alkaline phosphatase (ALP) from the ER to the vacuole is also indistinguishable in wild type and erv29Δ cells (Fig. 4C). Furthermore, erv29Δ cells show no growth inhibition, which would be expected if the transport of all proteins from the ER was significantly retarded (data not shown). These data suggest that Erv29p is required for the delivery of specific secretory proteins from the ER to the
substrates. The individual disruption of the involvement of other Erv proteins in degrading ERQC was also required for degrading PrA* (Fig. 5, degradation of CPY* to a small but significant extent. Erv14p was separated by centrifugation into soluble (Sol) and membrane pellet (Mem) fractions. The fractions were separated by SDS-PAGE and immunoblotted with either anti-HA, anti-Dpm1p, or anti-phosphoglycerol kinase antibodies. B, KHY271 (grp28Δ) cells expressing Ygr284p-HA (pAC530) or Ygr284p (untagged, pAC529) were fixed, converted to spheroplasts, and stained with either anti-HA or anti-Eug1p antibodies. The cells were viewed by Nomarski optics to observe cell morphology and epifluorescence microscopy using filter sets specific for 4',6'-diamidino-2-phenylindole to observe nuclear staining and rhodamine to observe either Ygr284p-HA or Eug1p staining.

![Fig. 3. Membrane association and localization of Ygr284p-HA.](image)

Golgi and likely acts during vesicle exit from the ER.

**Erv14p Is Also Required for the Efficient Degradation of Soluble ERQC Substrates**—The selectivity of the trafficking defect of correctly folded proteins from the ER to the Golgi complex in erv29Δ cells is comparable with that found in cells disrupted in other ERV genes. Given this common theme of forward transport defects shared by the erv mutants, we tested the involvement of other Erv proteins in degrading ERQC substrates. The individual disruption of ERV41, ERV46, ERV25, and EMP24 appeared to accelerate the degradation of CPY* at a small but significant extent. Erv14p was also required for degrading PrA* (Fig. 5, C and D) but not the membrane spanning ERQC substrates Vph1p* and Sec61-2p (Fig. 5, E and F).

**Transport between the ER and Golgi Is Necessary for Degradation of Soluble ERQC Substrates**—The requirement for Erv29p for efficient ERQC degradation suggests that transport between the ER and Golgi compartments is important in degrading soluble ERQC substrates. This was confirmed by observing the turnover kinetics of CPY*-HA in a strain containing the temperature-sensitive allele sec12-4 in which formation of transport vesicles exiting the ER is prevented at the restrictive temperature (43, 44). The turnover of CPY*-HA was severely inhibited in sec12-4 cells (MBY10-7a; t½ = 76 min) at the restrictive temperature relative to that in the SEC12 parent strain (ANY21; t½ = 34 min; Fig. 6, A and C). The arrest of protein exit from the ER was monitored by immunoprecipitation of CPY from the same radiolabeled cell extracts from which the CPY*-HA had been immunoprecipitated (Fig. 6B). Both MBY10-7a (sec12-4) and ANY21 (SEC12) cells degraded CPY*-HA at the same rate when grown at the permissive temperature (data not shown). The stabilizing effect of blocking ER exit was further examined by observing the turnover kinetics of PrA*-HA in a sec12-4 strain. The degradative rate of PrA*-HA was also severely inhibited in sec12-4 cells (MBY10-7a; t½ = 44 min) at the restrictive temperature compared with that in the SEC12 parent strain (ANY21; t½ = 13 min; Fig. 6, D and F). Again, the ER exit block was confirmed by monitoring CPY trafficking (Fig. 6E).

A role for ER-Golgi transport in the degradation of soluble ERQC substrates was further supported in experiments utilizing another secretory mutant, sec18-1. A temperature-sensitive allele of SEC18, encoding the yeast homologue of NSF, results in inhibition of membrane transport at multiple stages of the secretory pathway, including ER-Golgi transport (45), by blocking vesicle fusion with the target membrane (46). Determination of turnover kinetics of CPY*-HA in KHY286 (sec18-1) at the restrictive temperature also showed a strong stabilization of the protein compared with that in the parent SEC18 strain (JHRY20–2Ca; t½ = 26 min versus 82 min; Fig. 7, A and B).
Additional controls using CPY trafficking showed that the sec18 block in vesicular transport to the Golgi was imposed (data not shown).

**DISCUSSION**

Our search for new components involved in ER quality control led us to screen genes transcriptionally up-regulated by the UPR for their potential involvement. Through this approach we identified both YGR284c/ERV29 and ERV14 as necessary for a fully functional ERQC. Erv29p was found to be an integral membrane protein conserved among a number of species ranging from *S. cerevisiae* to *Homo sapiens*. Conserved within this group was the di-lysine motif -KKXX-COOH, which is thought to act as a Golgi to ER retrieval signal for membrane proteins by binding to α-COP1, a protein involved in retrograde transport from the Golgi to the ER (36, 47). The loss of Erv29p function observed when the di-lysine motif is no longer at the COOH terminus and the isolation of Erv29p in ER derived COPII vesicles (27) indicates that the di-lysine motif is functional, yet Erv29p displays a steady-state localization in the Golgi as observed by indirect immunofluorescence. This unexpected result of a protein containing a functional di-lysine motif yet displaying a likely steady-state distribution in the Golgi was also observed by Schroder and co-workers (41) who localized Emp47p, a di-lysine containing protein, to the Golgi, yet demonstrated that it cycled through the ER. It is now considered that variation in residues flanking the di-lysine motif,
labeled and CPY*-HA immunoprecipitated as described.

The ER exit of CPY or Gas1p (28). Cells lacking either a membrane spanning protein Axl2p, it too has little or no effect on the transport of the GPI-anchored protein Gas1p to the Golgi. The absence of Erv25p has a pronounced effect on the ER exit of invertase and Gas1p yet CPY is unaffected (48). To vary the steady-state distribution within the early secretory pathway (47), therefore, it may be that Erv29p possesses a weakened but functional di-lysine motif to ensure a Golgi localization while cycling through the ER.

The Role of Erv29p in ER-Golgi Transport—The deletion of various ERV genes causes varying effects on the transport of proteins to the Golgi. The absence of Erv25p has a pronounced effect on the transport of the GPI-anchored protein Gas1p to the Golgi yet has no effect on CPY trafficking (26), and although an ERV14 disruption blocks the ER exit of the membrane spanning protein Axl2p, it too has little or no effect on the ER exit of CPY or Gas1p (28). Cells lacking either ERV41 or ERV46 display no delay in CPY and Gas1p transport, although a cell-free assay found a modest but significant decrease in transport to the Golgi (27). The disruption of EMP24 retards the ER exit of invertase and Gas1p yet CPY is unaffected (48). Because of these differing effects, a number of the ERV and p24 family members have been proposed to participate in the selection of subsets of soluble cargo to exit the ER by potentially acting as cargo receptors. Erv29p is enriched in COPII vesicles exiting the ER (27), most likely cycles between the ER and Golgi and erv29Δ cells have a severe delay in delivery of a subset of cargo proteins (CPY and PrA) from the ER to the Golgi. These data suggest that, as proposed for other Erv proteins, Erv29p is likely involved at the ER exit stage for the loading of select cargo proteins into COPII vesicles for export, potentially in a direct role as a cargo receptor. Although cycling between the ER and Golgi, the steady-state Golgi localization of Erv29p might result from the swift incorporation of ER localized Erv29p into COPII vesicles for ER exit. Alternatively Erv29p might act in ER-Golgi transport by contributing to the fusion of ER-derived COPII vesicles with the Golgi apparatus, but such a role would predict that all proteins in COPII vesicles would be similarly inhibited in reaching the Golgi apparatus, which was not observed in erv29Δ cells. A third possibility is that Erv29p acts in cargo loading of retrograde vesicles exiting the Golgi bound for the ER as has been proposed for the p24 family of ER-Golgi cycling proteins that includes Erv25p and Emp24p (49). In this case there is possibly an unidentified protein that functions in the ER to load select cargo for ER export, and in erv29A cells this protein would not be efficiently packaged and returned to the ER, and its absence in the ER would retard forward transport of a set of cargo proteins.

The Role of Vesicular Transport in ERQC—Our analysis has found that efficient degradation of the soluble ERQC substrates CPY* and PrA* requires functional transport between the ER and Golgi. Surprisingly Finger and co-workers (9) found that blocking vesicular transport in a sec18-1 strain grown at the restrictive temperature had no effect on the degradation of either CPY* or PrA*. It may be that the long chase times employed by Finger and co-workers may have masked the role of ER-Golgi trafficking in ERQC, although close examination of the relevant figure (Fig. 5) in Finger et al. (9) shows what appears to be at least some stabilization of CPY* in the sec18 strain at the restrictive temperature. The degradation of the membrane spanning ERQC substrates Sec61-2p and Vph1p* does not appear to involve such transport as shown by the lack of stabilization of these proteins in erv14Δ or erv29Δ cells and the absence of an effect on unassembled Vph1p (30) and mouse MHC class I heavy chain (H-2Kα, Ref. 12) upon cessation of vesicular transport from the ER through a sec12 block.

Why would soluble and membrane spanning substrates be treated differently and why is transport between the ER and Golgi important for soluble ERQC substrates? It is quite likely that some ERQC components may play a specialized role in the degradation of either membrane-spanning or soluble substrates. For example, in returning an ERQC substrate to the translocon for retrotranslocation, membrane-spanning ERQC substrates are likely to re-enter the translocon from the lipid bilayer by a reversal of lateral gating (50), whereas soluble ERQC substrates in the ER would presumably re-enter the translocon from the ER lumenal aqueous phase, and this distinction may employ different ERQC components. In this case the specialized role likely involves the trafficking of soluble ERQC substrates to the Golgi prior to their return to the ER for retrotranslocation and degradation. The requirement for this trafficking of soluble substrates may be to receive a modification in the Golgi that, upon the return of the protein to the ER, is recognized by the ERQC machinery for degradation. Such a modification might increase the affinity of the substrate for the degradative machinery rather than be essential for degradation because some degradation still occurs in sec12 or sec18 cells at the restrictive temperature where ER-Golgi transport is completely blocked. We considered that the proposed Golgi-based modification acting as a degradation signal in the ER might be the addition of an α-1,6-mannose residue to N-linked glycoproteins such as CPY* and PrA* by the cis Golgi localized Och1p. Och1p is the α-1,6-mannosyltransferase that initiates N-linked mannos outer chain elongation in yeast (51) and is likely to be the first modifying Golgi enzyme to which an ERQC substrate would be exposed (52). Correctly folded proteins receiving such a modification would not again be exposed to the ER environment, but misfolded proteins would return to the ER possessing this modification and be degraded. However, disruption of OCH1 had no effect on the degradation kinetics of CPY* (data not shown), and therefore some other Golgi-based modification may be occurring. An alternative trafficking role involves ERQC substrates remaining in the ER, whereas the cycling of an as yet unidentified factor between the ER and Golgi is required for the degradation of soluble ERQC substrates.

The basis of the discriminating effect of ER-Golgi transport on the degradation of soluble and membrane-spanning ERQC substrates remains obscure. It is possible that the mobility of soluble substrates in the ER lumen may be greater than that of membrane spanning substrates in the lipid bilayer and are therefore more accessible to the ER exit sites so as to be readily available for exchange with the Golgi apparatus.
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Packaged into vesicles exiting the ER.

Although both Erv14p and Erv29p stabilize CPY* and PrA* turnover, they are unlikely to act in the same step in transport between the ER and Golgi as evidenced by the different affects on the forward transport of CPY and PrA observed in erv14Δ and erv29Δ cells. erv29Δ cells shows one of the strongest trafficking defects to date for the ERV and p24 family, whereas CPY transport kinetics appear unaffected in erv14Δ cells (28). In contrast to erv29, erv14 is not transcriptionally up-regulated by the UPR (13). In fact, apart from a slight up-regulation of erv41 and erv46, the remaining ERV and p24 family members are not significantly up-regulated by the UPR (13). If Erv29p participates in the cargo loading of soluble ERQC substrates in the ER, then what mechanism ensures that the substrate is repackaged in the Golgi for retrograde transport to the ER instead of being secreted to the cell surface? Erv14p may act in this required role, which would explain the stabilizing effects on CPY* and PrA* degradation and the near wild type rate of CPY forward transport in erv14Δ cells, yet it would not account for the block in ER exit of the integral membrane protein Axl2p (28). The unexpected acceleration of CPY* turnover mainly observed in erv14Δ and erv46Δ cells might be due to the activation of the UPR and associated up-regulation of the ERQC, or alternatively such proteins might act negatively to prevent the loading of certain cargo into COPII vesicles. The absence of such proteins may remove these constraints and allow the cargo loading of soluble ERQC substrates in the ER, then what happens?

In summary we have found that transport between the ER and Golgi is necessary for degrading soluble ERQC substrate degradation and that Erv29p might act as a receptor to load such proteins into COPII vesicles exiting the ER. Given the extent of homology between Erv29p and other members of the SURF4, including the di-lysine retrieval motif, it is likely that these Erv29p homologues perform a similar function in ER-Golgi transport, although the role they may play in ERQC, if any, awaits investigation. Furthermore it may be that the UPR-mediated transcriptional up-regulation of genes involved in vesicular transport between the ER and Golgi may not simply be a strategy to remove proteins from the ER but additionally acts to help degrade them.

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