A cytosolic degradation pathway, prERAD, monitors pre-inserted secretory pathway proteins

Tslil Ast, Naama Aviram, Silvia Gabriela Chuartzman and Maya Schuldiner

Dept. of Molecular Genetics, Weizmann Institute of Science, Rehovot 7610001, Israel

Correspondence should be sent to: maya.schuldiner@weizmann.ac.il

Abstract

The endoplasmic reticulum (ER) identifies and disposes of misfolded secretory pathway proteins through the actions of ER associated degradation (ERAD) pathways. It is becoming evident that a substantial fraction of the secretome transiently resides in the cytosol before translocating into the ER, both in yeast and in higher eukaryotes. To uncover factors that monitor this transient cytosolic protein pool, we carried out a genetic screen in *Saccharomyces cerevisiae*. Our findings highlighted a preinsertional degradation mechanism at the cytosolic leaflet of the ER, which we termed prERAD. prERAD relies on the concurrent action of ER localized ubiquitination and deubiquitination machineries, Doa10 and Ubp1. By recognizing C-terminal hydrophobic motifs, prERAD tags for degradation pre-inserted proteins that have remained on the cytosolic leaflet of the ER for too long. Our discoveries delineate a novel cellular safeguard, which ensures that every stage of secretory pathway protein biogenesis is scrutinized and regulated.
Introduction

Cellular quality control has an essential role in shaping and maintaining a functional cellular proteome (Princiotta et al., 2003; Schubert et al., 2000). One of the best characterized quality control triaging systems is that of the endoplasmic reticulum (ER), which comprises the central folding site of the secretory pathway. Disposal of misfolded ER proteins occurs via the ER associated degradation (ERAD) pathway (Brodsky and McCracken, 1997), which involves the recognition of misfolded substrates by the membrane embedded ERAD complexes, retro-translocation to the cytosol, ubiquitination and handoff to the proteasome for degradation (Hebert and Molinari, 2007; Meusser et al., 2005).

Two key pathways mediate ERAD for yeast secretory pathway proteins and they are centered around the Hrd1 and the Doa10 E3 ubiquitin ligases (Carvalho et al., 2006; Huyer et al., 2004; Vashist and Ng, 2004). The Hrd1 complex takes part in the degradation of proteins that bear misfolded lesions in the ER lumen or membrane, and is therefore also referred to as the ERAD-L/M pathway. The Doa10 complex recognizes transmembrane proteins with misfolded cytosolic domains, and is known as the ERAD-C pathway. Some auxiliary factors are common to both ERAD-L/M and ERAD-C, such as the Cdc48 complex, that generates the driving force which is required for membrane extraction in both pathways (Rabinovich et al., 2002 Ravid & Hochstrasser, EMBO J 2006).

To date, secretory pathway quality control has primarily been studied in the context of proteins that have successfully translocated into the ER. However, a large fraction of the secretome fails to engage the signal recognition particle (SRP), and is temporarily found in the cytosol prior to their translocation (Ast et al., 2013; Chen et al., 1993; Hessa et al., 2011; Shao and Hegde, 2011). We reasoned that additional cytosolic monitoring measures might be in effect for this stage of SRP-independent substrate biosynthesis. An unbiased, systematic genetic screen with an SRP-independent substrate revealed that this is indeed the case. If these proteins do not enter the ER, they are tagged for degradation on the cytosolic face of the ER leaflet in a pathway we term prERAD. prERAD relies on opposing forces of ubiquitination and deubiquitination machineries, ensuring that nascent SRP-independent proteins are
provided sufficient time to translocate but are cleared efficiently from the membrane if they fail to do so.

**Results and Discussion**

**An unbiased screen for pre-insertional degradation reveals a role for ERAD-C**

Proteins that undergo SRP-independent targeting to the ER must transiently reside in the cytosol. Since such secretory proteins contain hydrophobic targeting signals, should they remain within the cytosol, they would needlessly engage the cytosolic folding machinery, or possibly generate cytosolic aggregates. We therefore hypothesized that monitoring measures must be in place to clear the cytosol of proteins that have reached the ER membrane but have not translocated.

To uncover which proteins might mediate such pre-insertional degradation we performed an unbiased and systematic genetic screen in which we visualized the fluorescently tagged RFP-Gas1, an SRP-independent substrate of the glycosylphosphatidylinositol (GPI) family (Ast et al., 2013; Ng et al., 1996), on the background of mutations in 210 proteins that are affiliated with quality control (Fig. 1A). While the majority of mutations did not affect the localization of RFP-Gas1, we identified 9 mutations that generated a mislocalized pattern (Fig. 1B). These mutants could be grouped into 3 functional categories, affecting either the proteasome, the cytosolic arm of ER associated degradation (ERAD-C) or protein deubiquitination (DUBs).

To date, most ERAD studies have focused on its role in the quality control of secretory pathway proteins after they have successfully translocated (Hampton, 2002; Meusser et al., 2005). However, translocated Gas1 is completely luminal (Conzelmann et al., 1988), and would not be available to the cytosolic-sensing ERAD-C pathway. Moreover, the absence of the luminal arm of ERAD (ERAD-L) had no affect on the localization of RFP-Gas1, precluding an indirect effect on RFP-Gas1 localization in strains lacking functional ERAD pathways (Fig. S1). Therefore, we hypothesized that ERAD-C could be interacting with the pre-inserted, cytosolic, form of RFP-Gas1.
ERAD-C takes part in the cytosolic degradation of the SRP independent substrate Gas1

To measure which form of RFP-Gas1 was being stabilized by deletions in the ERAD-C pathway, we halted translation with cycloheximide and followed the remaining protein pool by western blot analysis (Fig. 2A). In WT cells, we found that the cytosolic form of RFP-Gas1 disappeared after 60 minutes of translational halt. When attenuating translocation, by utilizing the sec61-DAmP background, nearly all of RFP-Gas1 was found in the cytosol at time 0. Within 60 minutes this cytosolic protein pool had been cleared, indicating that a pre-insertional degradation pathway was indeed in affect. In Δdoa10 cells, the amounts of cytosolic RFP-Gas1 was both elevated and stabilized over the time course of 60 minutes, suggesting that ERAD-C is indeed responsible for tagging the cytosolic forms of RFP-Gas1 for degradation. Moreover, it appears that the translocation pathway is independent of the degradation pathway, as mature RFP-Gas1 is present in normal levels in Δdoa10 cells at the initial time point.

We hypothesized that Doa10 might degrade a subpopulation of the pre-inserted RFP-Gas1 for two reasons: (1) Degradation is the result of protein folding, as folded proteins have lost their translocation competence (2) Degradation is triggered by the cytosolic concentration or dwell time of the substrates. To differentiate between these two scenarios, we attenuated both translocation and degradation using a sec61-DAmP/Δdoa10 double mutant. Interestingly, we saw that at time 0, there is more mature protein when compared to the single mutant (sec61-DAmP). Furthermore, following 60 minutes of cycloheximide treatment, the majority of the cytosolic RFP-Gas1 had been trafficked onwards to the Golgi and cell membrane. Thus, it seems that the cytosolic pool of RFP-Gas1 is translocation-competent. When we imaged RFP-Gas1 in cells that were attenuated for both translocation and ERAD-C (Fig. S2), we saw that the absence of the E3 ubiquitin ligase, DOA10, altered RFP-Gas1 localization from cytosolic inclusion bodies to an ER pattern. This phenomenon is in line with findings that trafficking to some inclusion bodies depends on ubiquitination (Kaganovich et al., 2008). We therefore suggest that Doa10 can tag pre-inserted RFP-Gas1 for degradation, in a process that we have termed prERAD. prERAD does not appear to be based on the folding state or translocational competence of the substrate, but rather on its cytosolic-occupancy.
prERAD is triggered by the presence of hydrophobic C-terminal GPI anchoring sequences

We next set out to test the protein motifs that engage prERAD. We reasoned that hydrophobic motifs might flag SRP-independent proteins as mislocalized, should they be exposed in the cytosol. Our previous work has indicated that while all SRP-independent proteins have hydrophobic targeting signals in the form of a signal sequence (SS), the subgroup of GPI anchor proteins have an additional hydrophobic patch at their C-terminus, their GPI anchoring sequence (Ast et al., 2013). As our model SRP-independent protein Gas1 is one such GPI-anchored protein, we set out to examine which of its hydrophobic domains direct Doa10 dependent degradation. To this end, we tagged GFP with either the SS or GPI anchoring sequence of Gas1, and measured the stability of these fusion proteins in the cytosol. While both GFP and SS-GFP remained stable in the cytosol (Fig. 2B and S3A), regardless of the presence or absence of Doa10, the GFP-AS was degraded in the cytosol in a Doa10 dependent manner.

We next analyzed two additional fluorescently tagged GPI anchored proteins, Ccw14 and Tos6, in WT and ∆doa10 cells (Fig. 2C). In control cells, Gas1, Ccw14 and Tos6 all localized to the cell surface, while in the absence of Doa10, all three proteins accumulated intracellularly in addition to their normal localization at the cell surface. In contrast to Gas1, Ccw14 and Tos6 appear to aggregate in ∆doa10 cells, possibly due to a dosage difference, as they were expressed from a multi-copy plasmid while Gas1 was expressed from an endogenous locus. In addition, cycloheximide assays on YFP-Ccw14 confirmed the specific dependence on Doa10 and not on Hrd1 for cytosolic clearance (Fig. 2D). Thus, it appears that prERAD is indeed required for the elimination of pre-inserted GPI anchored proteins. In contrast, when we analyzed the cytosolic clearance of a protein bearing only a SS, CPY (Prc1), we could not detect any preinsertional degradation following a translational halt (Fig. S3B). These findings indicate that the prERAD pathway recognizes the hydrophobic C terminal patches displayed by GPI anchored proteins.

prERAD does not involve extraction from the ER membrane

The Cdc48 complex has previously been shown to generate the mechanical force needed to extract ERAD-C substrates from the membrane (Rabinovich et al., 2002).
We rationalized that since prERAD deals with pre-inserted proteins there would be no need for Cdc48 function in this pathway. To test this hypothesis, we expressed YFP-Ccw14 in temperature sensitive strains of the Cdc48 complex, namely cdc48<sup>ts</sup> and ufd1<sup>ts</sup>, grown at the restrictive temperature. Indeed, neither Cdc48 nor Ufd1 were required for the degradation of pre-inserted YFP-Ccw14, although their inactivation significantly attenuated the degradation of the misfolded luminal protein CPY* (Fig. 2C). The lack of dependence on the Cdc48 complex further demonstrates that these cytosolic proteins represent a <i>bona-fide</i> pre-insertional protein population. Moreover, these results indicate that only a subset of proteins comprising the ERAD-C complex is required to carry out prERAD.

**Effective prERAD depends on deubiquitination by Ubp1**

Finally, we set out to understand how the regulatory aspect of prERAD is maintained. A key force in any such pathway is a negative regulator, such as a deubiquitination enzyme, ensuring that translocation substrates are not immediately degraded. Indeed, of the 20 DUB deletion strains present in our screen, two affected RFP-Gas1 localization, <i>UBP1</i> and <i>UBP11</i>. We set out to further test the effect of these DUBs on RFP-Gas1, by overexpressing them (Fig. 3A). The overexpression of <i>UBP11</i> had no effect on the localization of RFP-Gas1, which makes sense in light of its mitochondrial localization (Fig. S4). Since the localization of Ubp11 was previously unknown, this raises the possibility that the RFP-Gas1 puncta generated in ∆<i>ubp11</i> were a secondary effect of an aberrant mitochondrial quality control overloading the cell and would be interesting to follow up on. In contrast, the overexpression of the ER localized <i>UBP1</i> (Schmitz et al., 2005) phenomimicked the loss of <i>DOA10</i>, resulting in accumulation of RFP-Gas1 on the ER surface. Indeed, the amounts of pre-inserted RFP-Gas1 were indeed elevated in cells overexpressing <i>UBP1</i>, as could be measured by western blot (Fig. 3B). In fact, the overexpression of <i>UBP1</i> elevated the fraction of preinserted/inserted RFP-Gas1 by over two fold, when compared to WT cells (p-value<0.025) (Fig. 3C). Thus, it appears that <i>DOA10</i> and <i>UBP1</i> mediate opposing forces in prERAD, working concurrently to fine-tune the amount of preinserted proteins at the ER surface.

While SRP-independent targeting and translocation is an efficient process (Fig. 4), the newly identified cytosolic degradation mechanism, prERAD, ensures the clearance of
pre-inserted proteins. prERAD is regulated by opposing forces of Doa10 dependent ubiquitination and Ubp1 mediated deubiquitination. It is tempting to suggest that the prERAD pathway monitors the extent of time a pre-inserted substrate has remained in the cytosol through ubiquitin chain length, along the lines of the calnexin/calreticulin cycle (Caramelo and Parodi, 2008). However, it is also possible that prERAD is activated when the preinserted protein has accumulated above a given threshold.

While Doa10 has been extensively implicated in the degradation of translocated malfolded ER proteins, there have also been reports of Doa10-dependant degradation of cytosolic and nuclear factors (Furth et al., 2011; Hochstrasser et al., 1991; Metzger et al., 2008; Ravid et al., 2006). Our findings add an additional group of substrates for this important E3 ligase, namely pre-inserted GPI-anchored proteins. It appears that Doa10 reacts to the cytosolic presence of the hydrophobic GPI anchoring sequence, since both various substrates bearing a GPI anchoring sequence, as well as the isolated sequence itself, demonstrated Doa10-dependent degradation.

As SRP-independent substrates must remain in a loosely folded confirmation prior to translocation (Tsukazaki et al., 2008; van den Berg et al., 2004), pre-insertional degradation cannot hinge on the distinction between folding and misfolding. To overcome this challenge, it seems that immediate degradation is attenuated by the action of an ER localized DUB, Ubp1, whose overexpression rescues the pre-inserted form of RFP-Gas1. It should be noted that the exact role of Ubp1 in prERAD remains elusive- while it is appealing to suggest that Ubp1 is directly deubiquitinating preinserted substrates, it is also possible that Ubp1 serves to regulate other factors (Bernardi et al., 2013; Liu et al., 2014). This balance between E3 ubiquitin ligase and DUBs is not unique to our system, and has been recently shown to fine-tune degradation in the case of CD4 (Zhang et al., 2013). Furthermore, in the case of preinserted tail anchored proteins, which bear a C-terminal transmembranal domain, antagonizing forces of protein ubiquitination and protection appear to be at play in the cytosol (Leznicki and High, 2012). Little is known regarding Ubp1- it has been shown to localize to both the ER and cytosol, and has been implicated in the stabilization of Ste6 and Golgi proteins (Poulsen et al., 2012; Schmitz et al., 2005). Thus, it seems that Ubp1 is involved in shaping and regulating the degradation of proteins within the secretory pathway.
The discovery of such a preinsertional degradation pathway raises questions as to when and to what extent it is required. Although the translocation of SRP-independent substrates is fairly efficient, upon ER stress, Gas1 will undergo cytosolic degradation if the unfolded protein response is not effectively activated (Liu and Chang, 2008). Similar findings in higher eukaryotes have revealed that during ER stress, the translocation of proteins bearing a mildly hydrophobic SS is attenuated, and these proteins are cleared from the cytosol in a proteasome-dependent manner (Kang et al., 2006; Rutkowski et al., 2007). Furthermore, it has been previously shown that in unstressed mammalian systems, a substrate's translocation efficiency can range from 95% to 60% (Levine et al., 2005). This would indicate that at any given time, the cell must identify and degrade a significant untranslocated cytosolic protein pool. While secretory pathway degradation has long been thought to take place predominantly within the ER following translocation, this emerging understanding of prERAD highlights the multiple checkpoints that must be in place at every stage of secretory pathway function.

Materials and methods

Yeast strains and strain construction

All yeast strains in this study are based on the BY4741 laboratory strain (Brachmann et al., 1998). General laboratory strains and strains created in this study are listed in Supplementary Table SII. Unless otherwise stated, strains harboring a deletion in a specific ORF were taken from the yeast deletion library (Giaever et al., 2002) and verified, while strains harboring a hypomorphic allele of an essential gene were taken from the DAmP (Decreased Abundance by mRNA Perturbation) library (Breslow et al., 2008). To construct the quality control mutation library, YeastMine was queried for genes that bear GO terms related to quality control (i.e. "Ubiquitination", "Degradation", "Proteasome", "Microautophagy"), resulting in a list of 210 genes found in Supplementary Table SI. In order to construct the SRP-independent query strain, RFP-Gas1, kindly provided by Howard Riezman, was inserted into the URA3 locus, including sequences from ~800bp upstream and ~300bp downstream to Gas1's ORF. Primers utilized in this study are listed in Supplementary Table SIII. To study their effect, cdc48-2 and ufd1-1, kindly provided by Randy Hampton, were grown at the restrictive temperature of 37°C for 4-6 hours prior to any experiment.
Yeast media and growth conditions

Cultures were grown at 30°C in either rich medium (1% Bacto-yeast extract (BD), 2% Bacto-peptone (BD) and 2% dextrose (Amresco)) or synthetic medium (0.67% yeast nitrogen base with ammonium sulfate and without amino acids (CondaPronadisa) and 2% dextrose (Amresco), containing the appropriate supplements for plasmid selection) (Sherman, 1991). When needed as selection markers, G418 (200 \( \cdot \)g/ml, Calbiochem) or Nourseothricin (Nat) (200 \( \cdot \)g/ml WERNER BioAgents) were added. In cases where G418 was required in a synthetic medium, yeast nitrogen base without ammonium sulfate (CondaPronadisa) was added and supplemented with Mono-Sodium Glutamate (Sigma) as an alternative nitrogen source.

Robotic Library Manipulations

All genetic manipulations were performed using Synthetic Genetic Array (SGA) techniques to allow efficient introduction of RFP-Gas1 into systematic yeast libraries. SGA was performed as previously described (Cohen and Schuldiner, 2010; Tong and Boone, 2006). Briefly, using a RoToR bench top colony arrayer (Singer Instruments, UK) to manipulate libraries in 384-colony high-density formats, haploid strains from opposing mating types, each harboring a different genomic alteration, were mated on rich media plates. Diploid cells were selected on plates containing all selection markers found on both parent haploid strains. Sporulation was then induced by transferring cells to nitrogen starvation plates. Haploid cells containing all desired mutations were selected for by transferring cells to plates containing all selection markers alongside the toxic amino acid derivatives Canavanine and Thialysine (Sigma-Aldrich) to select against remaining diploids. Each SGA procedure was validated by inspecting representative strains for the presence of RFP-Gas1 and for the correct genotype using check PCR.

High-throughput microscopy

Microscopic screening was performed using an automated microscopy set-up as previously described (Breker et al., 2013; Cohen and Schuldiner, 2010). Briefly, liquid cultures were grown O.N. in minimal media, in a shaking incubator (LiCONiC Instruments) in 30ºc. Cells grown to logarithmic stage and were transferred onto glass bottom 384-well microscope plates (Matrical Bioscience) coated with Concanavalin
A (Sigma-Aldrich). The strains were imaged with an automated inverted fluorescent microscopic ScanR system (Olympus), equipped with a cooled CCD camera. Images were acquired using a 60X air lens - excitation at 555/28 nm and emission at 617/73 nm. After acquisition, images were manually reviewed using the ScanR analysis program. Images were processed by the Adobe Photoshop CS3 program for slight contrast and brightness adjustments.

**Manual Microscopy**

Manual microscopy was performed using an Olympus IX71 microscope controlled by the Delta Vision SoftWoRx 3.5.1 software with X60 oil lens. Images were captured by a Photometrics Coolsnap HQ camera with excitation at 490/20 nm and emission at 528/38 nm (GFP) or excitation at 555/28 nm and emission at 617/73 nm (mCherry/RFP). Images were transferred to Adobe Photoshop CS3 for slight contrast and brightness adjustments.

**Protein extraction and detection**

Yeast protein extraction and cycloheximide assays were performed as previously described (Bhamidipati et al., 2005). In brief, about 2.5 O.D.600 of mid-logarithmic yeast cells were treated with 150µg/ml of cycloheximide (Sigma) for the length of time indicated. Cells were then harvested, and re-suspended in 10% TCA on ice for 20 min. Following this incubation, cells were centrifuged for 15 min. at 14,000 rpm at 4°C, and the supernatant was removed. The pellet was washed in acetone, and resuspended in 100µl of loading buffer (0.05 M Tris–HCl, pH 6.8, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.1% bromophenol blue). 100µl of glass beads (Scientific Industries Inc.) were added to the loading dye, and the samples were bead-beaten for 5 min. at 4°C. The samples were then incubated at 95°C for 5 min., and centrifuged for 5 min. at 8,000rpm at RT. 20µl from the supernatant of the samples was resolved on 7.5 or 10% polyacrylamide gels, and probed with α-RFP (ab62341, Abcam), α-GFP (ab290, Abcam), α-HistoneH3 (ab1791, Abcam), α-HA (MMS-101P, Covance), α-CPY (ab113685, Abcam) or α-PGK (459250, Life Technologies). Secondary antibodies consisted of goat α-rabbit or α-mouse conjugated to IRDye800 or to IRDye680 (LI-COR Biosciences), and were scanned for infrared signal using the Odyssey Imaging System (LI-COR Biosciences). Protein amount was quantified.
using the Image Studio software (LI-COR Biosciences), and was tested for statistically significant variation using Student's t-test.

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Author Contributions

MS, TA, NA and SGS designed and performed the experiments as well as analyzed the data. MS and TA wrote the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Figure Legends

Figure 1- A screen for preinsertional degradation effectors of SRP-independent proteins reveals components of the proteasome, ERAD-C and DUBs

(A) To uncover pathways that take part in the preinsertional degradation of SRP-independent substrates, a genetic screen was carried out in the budding yeast Saccharomyces cerevisiae. A fluorescently tagged model SRP-independent protein, RFP-Gas1, was crossed against a quality control mutation library, utilizing the synthetic genetic array (SGA) technique. This custom library was visually screened for mutants in which RFP-Gas1 was mislocalized within the cell. (B) Representative images of control (WT) and mutation backgrounds that affected RFP-Gas1 localization (x60 lens). These nine proteins can be grouped into three functional categories, affecting either proteasome, ERAD-C or deubiquitinating enzyme (DUB) function.
Figure 2- prERAD mediates the preinsetional degradation of GPI anchored proteins by employing Doa10, but not Hrd1 or the Cdc48 complex

(A) The degradation of RFP-Gas1 following translational shut-off (by addition of cycloheximide) was analyzed by western blot with αRFP. The cytosolic RFP-Gas1 pool is efficiently degraded when translocation is attenuated in sec61-DAmP. In cells lacking Doa10, this cytosolic pool is stable. When both translocation and degradation are attenuated, the cytosolic protein can mature through the few translocons present. Histone was used as a loading control. (B) The cytosolic stability of GFP tagged with Gas1’s SS or GPI anchoring sequence was tested by western blot with αGFP. While SS-GFP is stable in the cytosol, GFP-AS is degraded in the cytosol in a Doa10 dependent manner. PGK was used as a loading control. (C) Three fluorescently tagged GPI anchored proteins, Gas1, Ccw14 and Tos6 were imaged in either control or Δdoa10 strains (lens x60). While in the WT background, all three proteins are localized to the cell surface, they undergo accumulation in cytosolic forms in the Δdoa10 strain. (D) The degradation of YFP-Ccw14 following translational shut-off was analyzed by western blot with α-GFP. prERAD of cytosolic YFP-Ccw14 is independent of the ERAD-M/L (Δhrd1) pathway, as well as Cdc48 and Ufd1 (cdc48ts and ufd1ts, respectively). As a control, the stability of CPY*-HA, a known substrate of Hrd1, Cdc48 and Ufd1 was analyzed. Histone was used as a loading control.

Figure 3- Immediate prERAD degradation is attenuated by the ER bound DUB-UBPI.

(A) RFP-Gas1 was imaged in WT strain, or strains overexpressing UBPI or UBPI1 (lens x100). The normal cell surface localization of RFP-Gas1 is not altered when overexpressing UBPI1. However, upon overexpression of UBPI, RFP-Gas1 is found in an additional ER pattern, which co-localizes with the ER marker, Scs2-GFP. (B) RFP-Gas1 was analyzed by western blot in strains overexpressing UBPI or UBPI1 with α-RFP. Overexpression of UBPI stabilized the cytosolic fraction of RFP-Gas1. Histone was used as a loading control. (C) The fraction of cytosolic/mature amounts of RFP-Gas1 was quantified in a WT strain, or strains overexpressing UBPI or UBPI1 as detected by WB (normalized mean ± SEM depicted). While the overexpression of UBPI1 does not alter this ratio, the overexpression of UBPI significantly raises this balance by over 2 fold (*p-value<0.025).
Figure 4- Schematic model of prERAD.

Should a protein bearing a GPI anchor sequence (AS) fail to translocate in a timely manner, it will be engaged by the E3 ligase, Doa10. The presence of deubiquitinating machinery allows this SRP-independent substrate to elude immediate degradation and undergo translocation. However, should the substrate loiter in the cytosol, its chances to be degraded by the proteasome will increase, linking cytosolic occupancy with degradation.

References


A

SRP independent query

RFP-Gas1

Quality Control Mutation Library

Δxxx

Visualized Phenotypes

Wild Type

Cytoplasmic Puncta

Endoplasmic Reticulum

Synthetic Genetic Array

Microscopic Analysis

SRP independent query in mutant background

B

Proteasome

WT

Δirc25

Δpoc4

pup1-DAmp

Δsem1

ERAD-C

Δdoa10

Δubc7

Δcue1

Ubiquitin Proteases

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prERAD-
preinsertional protein clearance

SRP independent translocation

Degradation

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Cytosol

Sec62/3 complex

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