



**HAL**  
open science

## Quality control in the secretory pathway

Efrat Rabinovich, Dana Bussi, Ilana Shapira, Guy Alalouf, Carni Lipson,  
Yechiel Elkabetz, Michael Glickman, Monika Bajorek, Shoshana Bar-Nun

► **To cite this version:**

Efrat Rabinovich, Dana Bussi, Ilana Shapira, Guy Alalouf, Carni Lipson, et al.. Quality control in the secretory pathway. Harefuah, 2006, 8 (4), pp.238-242. hal-01603035

**HAL Id: hal-01603035**

**<https://hal.science/hal-01603035>**

Submitted on 29 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

## Quality Control in the Secretory Pathway

Efrat Rabinovich PhD<sup>1</sup>, Dana Bussi MSc<sup>1</sup>, Ilana Shapira DMD<sup>1</sup>, Guy Alalouf MSc<sup>1</sup>, Carni Lipson BSc<sup>1</sup>, Yechiel Elkabetz PhD<sup>1</sup>, Michael Glickman PhD<sup>2</sup>, Monica Bajorek MSc<sup>2</sup> and Shoshana Bar-Nun PhD<sup>1</sup>

<sup>1</sup>Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel

<sup>2</sup>Department of Biology, Technion, Haifa, Israel

**Key words:** ubiquitin, proteasome, AAA-ATPases, ERAD, p97/Cdc48p, Rpt subunits

IMAJ 2006;8:238–242

The intracellular fate of secretory immunoglobulin M and its  $\mu$ s heavy chain is altered during differentiation of B lymphocytes. We have shown that sIgM is efficiently secreted from plasma cells but is retained and rapidly degraded from B cells [1-3]. The degradation mechanism of retained proteins was elucidated by studies on the  $\Delta$ F508 of the cystic fibrosis transmembrane conductance regulator [4,5]. It became evident that proteins that are retained in the endoplasmic reticulum are tagged by ubiquitin and eliminated by the 26S proteasome. This pathway, known as the ER-associated degradation or ERAD, is a quality control mechanism, because it eliminates misfolded proteins and orphan subunits of oligomeric proteins. In addition, the ERAD pathway is responsible for the destruction of unwanted proteins as the  $\mu$ s in B cells. We have shown that  $\mu$ s is a *bona fide* luminal ERAD substrate. In B cells,  $\mu$ s is tagged by ubiquitin and degraded by the proteasome [2,6].

We are studying *cis*-acting motifs that act as retention signals and degrons and searching for *trans*-acting cellular factors that play a role in conferring retention and degradation. The best-characterized *cis*-acting motif is the  $\mu$ stpCys, the carboxy-terminal tailpiece of the  $\mu$ s that distinguishes this secretory heavy chain from the membrane  $\mu$ m form. It has been shown that  $\mu$ stpCys, comprising the unique 20 residues of the  $\mu$ s and its penultimate Cys575, are necessary for the retention of  $\mu$ s in B cells [7]. As shown in Figure 1, the  $\mu$ stpCys is also sufficient to confer ER retention onto reporter proteins, when fused to thyroid peroxidase (TPO) from which the transmembrane segment was deleted [Figure 1A] or to yellow fluorescent protein which is led to the secretory pathway by hen lysozyme signal sequence (ssYFP) [Figure 1B]. Clearly, when the penultimate Cys is replaced by Ser, the TPO $\mu$ stpSer is secreted to the medium [Figure 1A] and the ssYFP $\mu$ stpSer reaches the Golgi [Figure 1B]. Moreover, this  $\mu$ stpCys-conferred ER retention is not restricted to B cells, as this phenomenon takes place in the non-lymphoid COS-7 or HEK293 cells [Figure 1].

The  $\mu$ stpCys acts as a 'degron', as it confers degradation onto TPO [Figure 1 A,C and D] and ssYFP [Figure 1E]. Moreover, the  $\mu$ stpCys-conferred degradation is proteasomal, as TPO $\mu$ stpCys [Figure 1D] as well as ssYFP $\mu$ stpCys [Figure 1E] are stabilized in

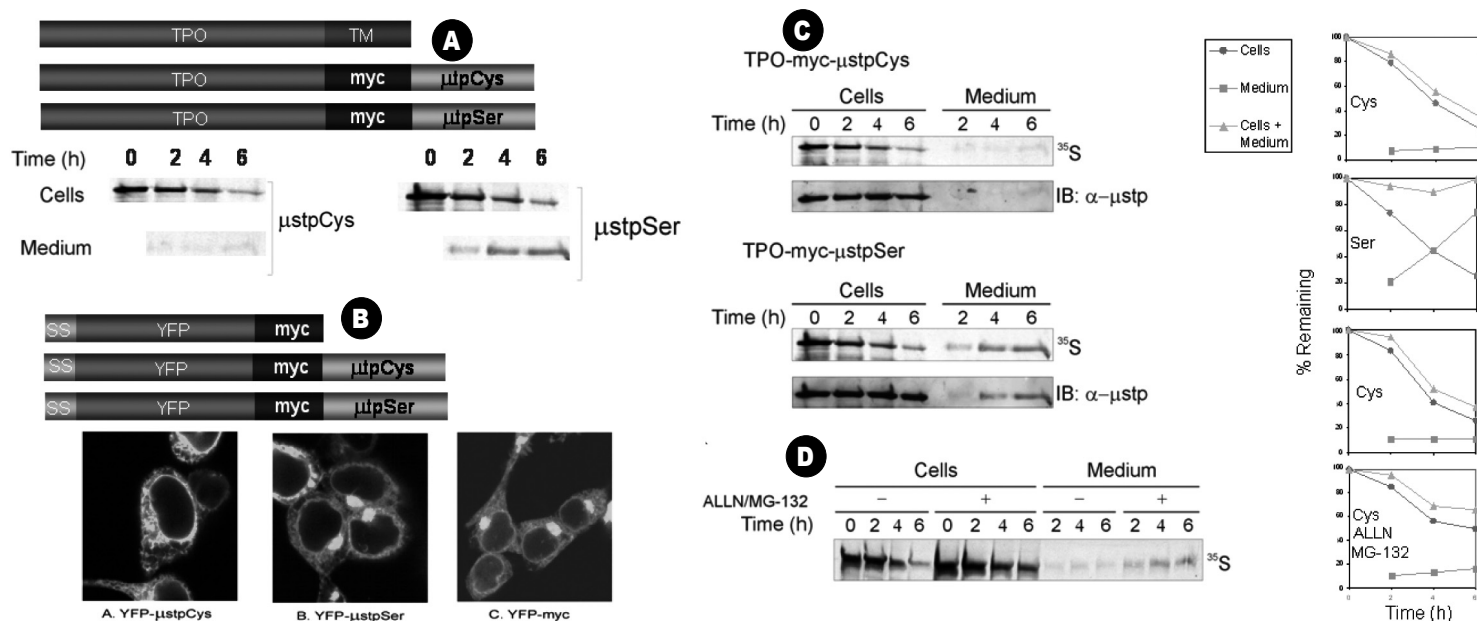
the presence of proteasome inhibitors. Again, this degradation occurs in the non-lymphoid HEK293 cells and interestingly, when ssYFP $\mu$ stpCys is expressed in the 38C B cells, its degradation rate resembles the turnover of the endogenous  $\mu$ s heavy chain [Figure 1E]. Acting as a degron, the  $\mu$ stpCys most likely recruits the ubiquitination machinery to the otherwise stable reporter proteins. Nonetheless, the ubiquitin tag is not necessarily conjugated to the  $\mu$ stpCys, because degradation of the TPO $\mu$ stpCys is indifferent to K-to-R substitution of the only Lys in the  $\mu$ stp [Figure 2A]. The half-life of 174 minutes measured for the wild-type chimera remains 178 minutes for the KR mutant [Figure 2C]. Hence, it indicates that either the ubiquitin is always conjugated to other Lys residues located in the reporter protein, or the ubiquitin conjugation is a promiscuous process that allows additional Lys residues to take over if the preferred K is mutated. Moreover, Asn563 in the  $\mu$ stp is a glycosylation site that in the case of TPO $\mu$ stpCys is the most C-terminal of an additional four putative glycosylation sites. N-glycans play an important role in ER quality control and in ERAD [8,9] and it has been shown for the yeast ERAD substrate carboxypeptidase Y\* that the most C-terminal of four N-glycans is necessary and sufficient to direct this protein to degradation [10,11]. However, the N-to-Q substitution in the TPO $\mu$ stpCys not only does not hamper degradation, but in fact accelerates the turnover of the NQ chimera [Figure 2B], shortening the half-life from 174 to 112 minutes [Figure 2C].

In our search for *trans*-acting factors that participate in ERAD, we used the  $\mu$ s heavy chain as a bait and co-precipitated it with p97 [12]. The connection of this cytosolic AAA-ATPase to ERAD was suggested from the co-immunoprecipitation of p97 with the rapidly degraded  $\mu$ s in B cells but not with the same  $\mu$ s in plasma cells in which this heavy chain is a stable, secreted protein [12]. To unequivocally show the involvement of p97/Cdc48p in ERAD, we used yeast strains that harbor conditional mutants of Cdc48p, the p97 ortholog, as p97 is an essential protein [12]. Several groups have shown that p97/Cdc48p is an essential ERAD component [13-16]. Here we show that in mammalian cells, expression of excess p97 does not affect the turnover of ssYFP $\mu$ stpCys [Figure 2D], suggesting that this abundant protein is not limiting. However, expression of equimolar amounts of the K524A p97 mutant stabilizes the ssYFP $\mu$ stpCys [Figure 2D], suggesting that the ATP binding is essential for the function of this AAA-ATPase in ERAD.

sIgM = secretory immunoglobulin M

ER = endoplasmic reticulum

TPO = thyroid peroxidase



**Figure 1.** The  $\mu\text{stpCys}$  acts as a retention signal and as a 'degron' HEK293 were transfected with vectors encoding myc-tagged TPO- $\mu\text{stpCys}$  or TPO- $\mu\text{stpSer}$  (schematic constructs are presented) [A,C], TPO- $\mu\text{stpCys}$  [D], myc-tagged ssYFP, ssYFP- $\mu\text{stpCys}$  or ssYFP- $\mu\text{stpSer}$  (schematic constructs are presented) [B], or ssYFP- $\mu\text{stpCys}$  [E, right panel]. A vector expressing ssYFP- $\mu\text{stpCys}$  was also introduced to 38C B cells [E, left panel]. Cells were pulse-labeled with  $^{35}\text{S}$ -Met/Cys and chased for the indicated time without [A,C] or with (+) or without (-) ALLN and MG-132 [D,E]. Cells and medium were separated, and substrates were immunoprecipitated (IP) by an anti-myc antibody [A,C,D], or from cells only by anti-myc [E, right panel] or by a combination of anti-myc and anti- $\mu$  antibodies [E, left panel]. Immunoprecipitates were resolved by SDS-PAGE, electroblotted and blots were exposed to autoradiography ( $^{35}\text{S}$ ) [A,C,D,E] and then probed (IB) with an anti- $\mu\text{stp}$  antibody ( $\alpha\text{-}\mu\text{stp}$ ) [C, lower panels]. For TPO- $\mu\text{stpCys/Ser}$ , the remaining amounts – cells (circles), medium (squares) or their sum (triangles) – were calculated from densitometry as the percentage of the level at the beginning of chase (100%) and presented in graphs [C,D]. Alternatively, YFP was followed by confocal fluorescent microscopy [B].

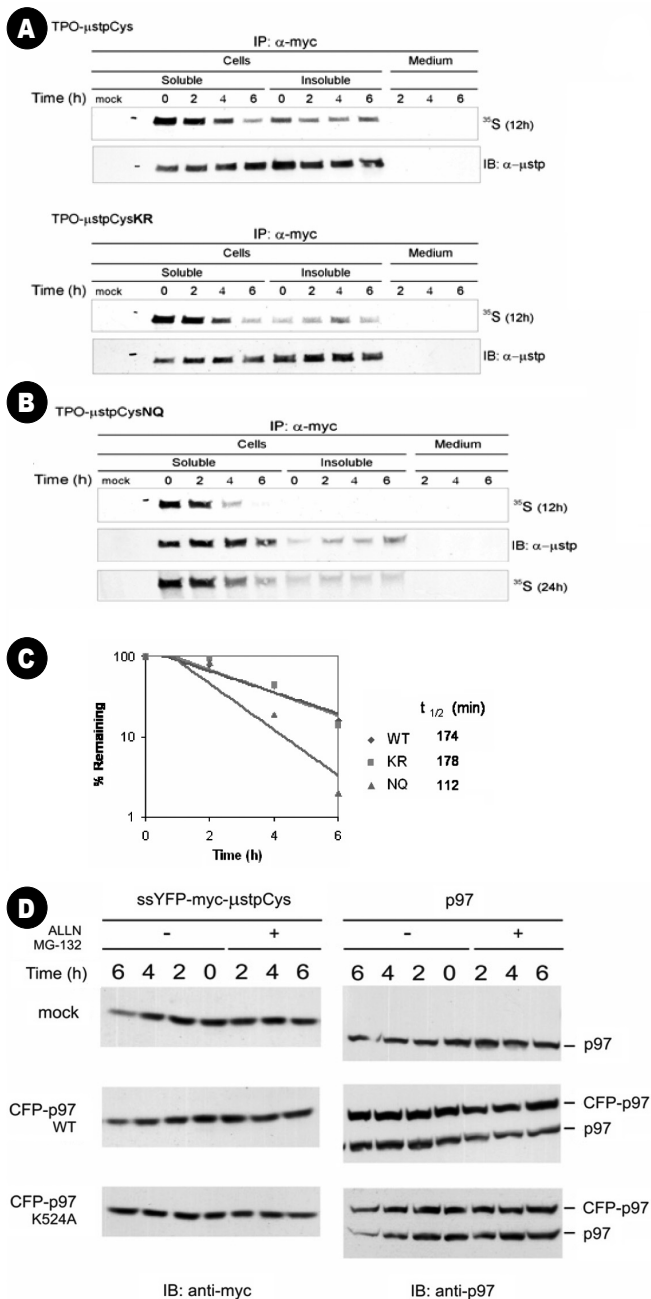
Being a cytosolic ATPase, we hypothesized that p97/Cdc48p provides the driving force for dislocating ERAD substrates from the ER back to the cytosol, where the ubiquitin-proteasome system resides. This notion has been supported by activation of the unfolded protein response in the *cdc48* mutant at the non-permissive temperature [12]. Subsequently, we dissected the dislocation of the luminal ERAD substrate CPY\* into two consecutive steps – passage across the ER membrane and release to the cytosol – and showed that Cdc48p is essential for the passage [17]. The possibility that p97/Cdc48p provides the driving force for dislocation is in agreement with the view that AAA-ATPases are molecular machines that generate mechanical force by undergoing conformational changes upon nucleotide binding [18].

Additional AAA-ATPases that could play a role in ERAD are located at the base of the 19S regulatory particle of the proteasome. Known in yeast as Rpt subunits, we have taken advantage of strains in which a single Rpt is mutated at the conserved ATP binding K [19]. K-to-R substitution has been introduced to Rpt3, Rpt4, Rpt5 and Rpt6; K-to-S substitution has been introduced

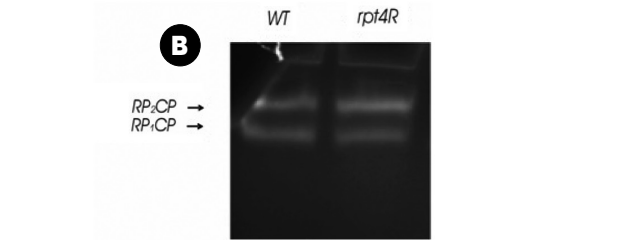
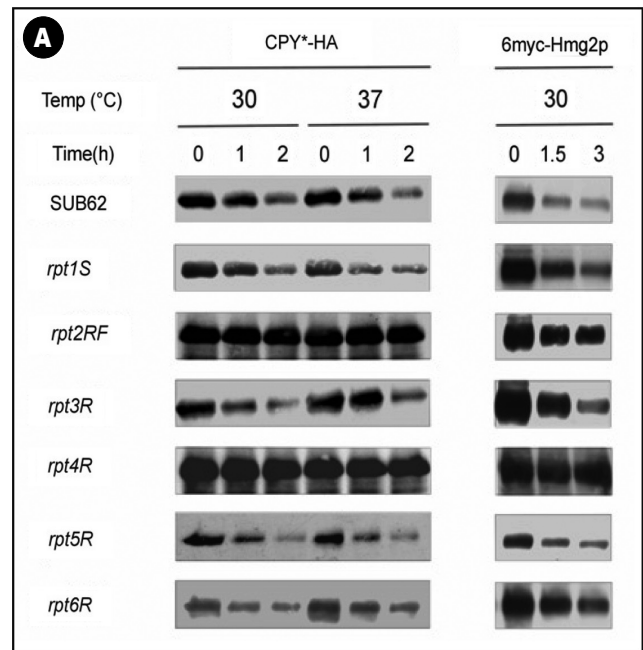
to Rpt1; K-to-R substitution accompanied by a second site S-to-F substitution has been introduced to Rpt2. It appears that from the six 19S Rpt subunits, Rpt2p and Rpt4p are the only AAA-ATPases required for ERAD of either the luminal substrate CPY\*-HA or the membrane substrate  $\delta\text{myc-Hmg2p}$  [Figure 3A]. However, striking functional differences between Rpt2p and Rpt4p are revealed by monitoring the proteasome activity by zymography and visualizing the proteasome in non-denaturing PAGE upon incubation with the fluorogenic substrate Suc-LLVY-AMC [19]. The Rpt2RF-containing proteasome is inactive in this *in vitro* assay, probably due to gated access to the proteolytic chamber within the catalytic particle [19]. Conversely, the Rpt4R-containing proteasome is structurally stable and proteolytically active in this *in vitro* assay [Figure 3B]. This suggests that while Rpt2p is involved in the degradation of every proteasomal substrate, Rpt4p could preferentially participate in the degradation of ERAD substrates.

A step that is unique to ERAD substrates is their dislocation; we therefore speculate that Rpt4p contributes to dislocation. Indeed, cell fractionation and protease protection assay show that the dislocation of the luminal ERAD substrate CPY\*-HA is hampered in the *rpt4R* strain but not in the *rpt2RF* strain [Figure 4 A and B]. Using cell fractionation and protease protection assay,

CPY\* = carboxypeptidase Y\*



**Figure 2.** The  $\mu$ stpCys is not ubiquitinated, its N-glycosylation decelerates degradation, and expression of p97(K524A) stabilizes ssYFP $\mu$ stpCys. HEK293 were transfected with an empty vector (mock), with vectors encoding myc-tagged TPO- $\mu$ stpCys (WT) or TPO- $\mu$ stpCysKR in which the only K in the  $\mu$ stp was replaced by R [A], TPO- $\mu$ stpCysNQ in which the only N in the  $\mu$ stp was replaced by Q [B], or combinations of myc-tagged ssYFP $\mu$ stpCys and vectors encoding CFP-p97 wild-type (WT) or the K524A mutant [D]. Cells were pulse-labeled with  $^{35}$ S-Met/Cys and chased for the indicated time. Cells and medium were separated, cells were lysed and substrates were immunoprecipitated (IP) by an anti-myc antibody. Immunoprecipitates were resolved by SDS-PAGE, electroblotted and blots were exposed to autoradiography ( $^{35}$ S) [A,B] and then probed (IB) with an anti- $\mu$ stp antibody ( $\alpha$ - $\mu$ stp) [A,B]. The remaining amounts of WT TPO- $\mu$ stpCys (romboids), TPO- $\mu$ stpCysKR (squares) or TPO- $\mu$ stpCysNQ (triangles) were determined from densitometry as the percentage of the level at the beginning of chase (100%), plotted on a semi-logarithmic graph [C], and half-life values were calculated. Alternatively, at the indicated time after cycloheximide addition, degradation of ssYFP $\mu$ stpCys was monitored by immunoblotting with an anti-myc antibody [D, left panels] and blots were re-probed with an anti-p97 antibody [D, right panels]. When indicated, the proteasome inhibitors ALLN and MG-132 were also added.

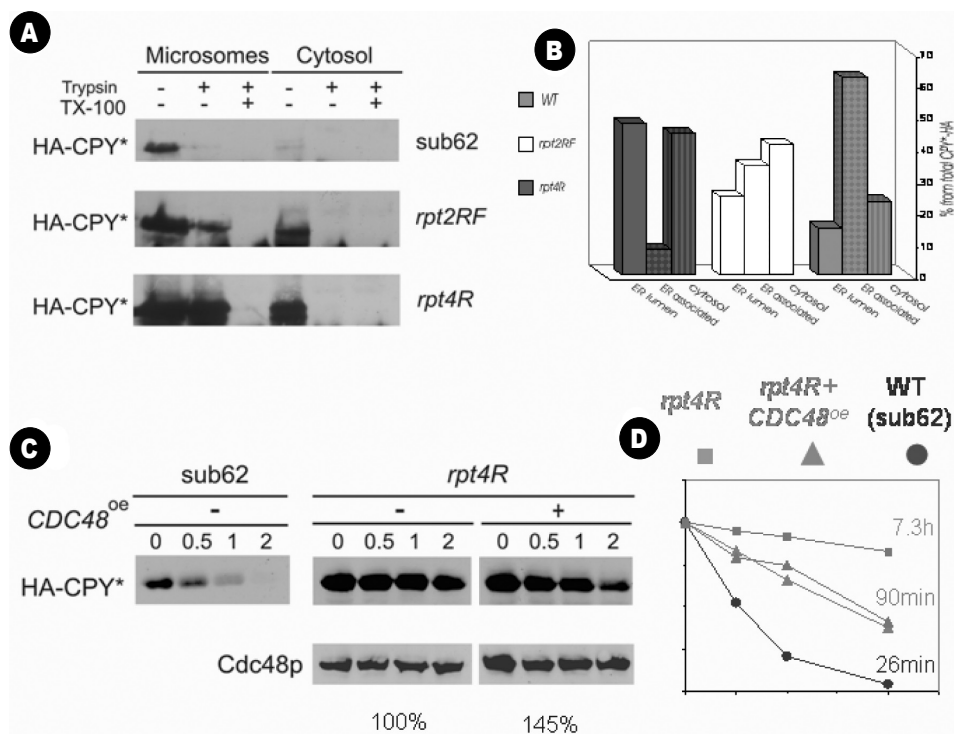


**Figure 3.** *rpt* mutants – stability, *in vitro* proteolytic activity and *in vivo* turnover of luminal and membrane ERAD substrates.

[A] Degradation of CPY\*-HA (left panels) and 6myc-Hmg2p (right panels) was followed in strains SUB62 (wild-type RPT), *rpt1S*, *rpt2RF*, *rpt3R*, *rpt4R*, *rpt5R* and *rpt6R* (*rpt* mutants). Following pre-incubation for 2 hours at the indicated temperatures, cycloheximide (150  $\mu$ g/ml) was added and cells (2 OD<sub>600</sub>) were collected at the indicated time points. Cells were dissolved and total cellular proteins were resolved by reducing SDS-PAGE and electroblotted onto nitrocellulose. Blots were probed with anti-HA (left panels) or anti-myc (right panels) mouse antibodies followed by HRP-conjugated anti-mouse IgG, and the HRP was visualized by ECL.

[B] Post-nuclear supernatant (PNS) was prepared from SUB62 (WT) and *rpt4R* mutant strain by disrupting yeast cells with glass beads in ice-cold Tris-HCl buffer. PNS was cleared by centrifugation (14,000 rpm, 10 minutes, 4°C) and equal amounts of protein were loaded onto non-denaturing PAGE supplemented with 0.5 mM ATP. Proteasome bands were visualized by peptidase activity assay using the fluorogenic substrate Suc-LLVY-AMC. The slower migrating band represents the doubly capped proteasome (RP<sub>2</sub>CP) and the faster migrating band the singly capped proteasome (RP<sub>1</sub>CP).

we show that CPY\*-HA is similarly trapped within the ER lumen in either yeast strain expressing the temperature-sensitive *cdc48* at restrictive temperature [17] or the *rpt4R* strain [Figure 4 A and B], suggesting that Cdc48p and Rpt4 participate in the passage of CPY\*-HA across the ER membrane. Hence, we have hypothesized that excess Cdc48p would be able to suppress the ERAD defects in the *rpt4R* strain. Indeed, over-expression of Cdc48p from a plasmid to 145% of its initial level accelerates the degradation of CPY\*-HA in the *rpt4R* strain by about fivefold [Figure 4C]. While the half-life of CPY\*-HA in the *rpt4R* mutant was 17 times longer than that in the wild-type strain, extending from 26 minutes to



**Figure 4.** Rpt4 is involved in dislocation of CPY\*-HA and over-expression of Cdc48p suppresses ERAD deficiency in *rpt4R*.

**[A]** Spheroplasts from SUB62 (WT strain) (upper panel), *rpt2RF* (middle panel) or *rpt4R* (lower panel) expressing CPY\*-HA were disrupted in sorbitol buffer, and post-nuclear supernatants (PNS) were treated with (+) or without (-) trypsin together (+) or without (-) Triton-X100. After trypsin inactivation, samples were dissolved, CPY\*-HA was immunoprecipitated with a mouse anti-HA antibody, resolved by SDS-PAGE, immunoblotted and blots were probed with anti-HA.

**[B]** Blots were quantified by densitometry and the diagrams represent the distribution of CPY\*-HA in the ER lumen (microsomes treated with trypsin), at the cytosolic face of the ER membrane (subtracting microsomes treated with trypsin from untreated microsomes) or in the cytosol (untreated cytosol), as percent of total CPY\*-HA (sum of untreated microsomes and untreated cytosol).

**[C]** A plasmid for expression of CPY\*-HA together (+) or without (-) a plasmid for expression of Cdc48p was introduced into SUB62 (WT) or *rpt4R* strains. Degradation of CPY\*-HA was followed by addition of cycloheximide (150  $\mu$ g/ml) and samples (2 OD<sub>600</sub>) were collected at the indicated time points. Cells were dissolved and total cellular proteins were resolved by reducing SDS-PAGE and electroblotted onto nitrocellulose. Blots were probed with a rabbit anti-Cdc48 antibody (lower panel) followed by HRP-conjugated anti-rabbit IgG, and re-probed with a mouse anti-HA antibody (upper panel) followed by HRP-conjugated anti-mouse IgG, and HRP was visualized by ECL. Relative amounts of Cdc48p were quantified by densitometry.

**[D]** Blots were quantified by densitometry and the plot represents the decay of CPY\*-HA as remaining protein calculated as a percentage of its level at the time of cycloheximide addition (100%). Half-life values are presented for WT (circles), *rpt4R* (squares) and *rpt4R*+*CDC48* (triangles).

7.3 hours, increased expression of Cdc48p results in half-life shortening to only 85–95 minutes [Figure 4D].

What could be the inter-relationships between Cdc48p and Rpt4p with respect to the ERAD-specific dislocation process? In principle, Cdc48p and Rpt4p may operate in parallel, performing similar but independent functions. The parallel model is corroborated by the binding of the proteasome as well as of p97/Cdc48p to the ER membrane, because mechanistically, association with the ER membrane is anticipated to facilitate the function of AAA-ATPases in pulling substrates out of the ER. Although p97/Cdc48p and

the proteasome are largely free in the cytosol, their small ER-bound subpopulations might be the one relevant to ERAD, as suggested by the exclusive interaction of the luminal ERAD substrate  $\mu$ s with these small ER-bound subpopulations [17]. Moreover, it has been reported that binding of proteasomes to ER membranes is salt-sensitive, ATP-dependent, and mediated by the high affinity binding of the Rpt-containing base of the 19S regulatory particle [20].

Alternatively, Rpt4p and Cdc48p may act sequentially, where Cdc48p binds ERAD substrates and delivers them to the 26S proteasome in a fashion that depends on the ATPase activity of Rpt4p. Indeed, ATP hydrolysis has been shown to modulate the association of many proteins with the 26S proteasome [21]. In this

model, the proteasome binding to the ER membrane is irrelevant to its role in ERAD, whereas the binding of Cdc48p to the ER membrane is obligatory. Several lines of evidence support the notion that the p97/Cdc48p that operates in ERAD is ER-bound. First, the luminal ERAD substrate  $\mu$ s pulls down only the small subpopulation of the ER-bound p97 [17]. Second, p97 is bound to the ER membrane by a membrane protein complex containing VCP-interacting membrane protein and Derlin-1, the mammalian homologue of Der1 and Dfm1 and the putative dislocation pore protein [22]. Third, the UBX domain protein Ubx2/Sel1, an integral ER membrane protein, is required for ERAD in yeast because it recruits Cdc48p to the ER and together they interact with Der1 and Dfm1 [23,24]. Finally, deletion of the PKC1 gene impairs ERAD and causes accumulation of CPY\* in the ER lumen, most probably due to Cdc48p mislocalization [25].

## References

1. Amitay R, Bar-Nun S, Haimovich J, Rabinovich E, Shachar I. Post-translational regulation of IgM expression in B lymphocytes. *J Biol Chem* 1991;266:12568–73.
2. Amitay R, Shachar I, Rabinovich E, Haimovich J, Bar-Nun S. Degradation of secretory IgM in B lymphocytes occurs in a post-endoplasmic reticulum compartment and is mediated by a cysteine protease. *J Biol Chem* 1992;267:20694–700.
3. Shachar I, Amitay R, Rabinovich E, Haimovich J, Bar-Nun S. Polymerization of secretory IgM in B lymphocytes is prevented by a preceding targeting to a degradation pathway. *J Biol Chem* 1992;267:24241–7.
4. Ward CL, Omura S, Kopito RR. Degradation of CFTR by the ubiquitin-proteasome pathway. *Cell* 1995;83:121–7.
5. Jensen TJ, Loo MA, Pind S, Williams DB, Goldberg AL, Riordan JR. Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. *Cell* 1995;83:129–35.

- Elkabetz Y, Kerem A, Tencer L, Winitz D, Kopito RR, Bar-Nun S. Immunoglobulin light chains dictate vesicular transport-dependent and -independent routes for IgM degradation by the ubiquitin-proteasome pathway. *J Biol Chem* 2003;278:18922–9.
- Sitia R, Neuberger M, Alberini C, et al. Developmental regulation of IgM secretion: the role of the carboxy-terminal cysteine. *Cell* 1990;60:781–90.
- Helenius A, Aebi M. Intracellular functions of N-linked glycans. *Science* 2001;291:2364–9.
- Ellgaard L, Helenius A. Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 2003;4:181–91.
- Kostova Z, Wolf DH. Importance of carbohydrate positioning in the recognition of mutated CPY for ER-associated degradation. *J Cell Sci* 2005;118:1485–92.
- Spear ED, Ng DT. Single, context-specific glycans can target misfolded glycoproteins for ER-associated degradation. *J Cell Biol* 2005;169:73–82.
- Rabinovich E, Kerem A, Frohlich KU, Diamant N, Bar-Nun S. AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. *Mol Cell Biol* 2002;22:626–34.
- Bays NW, Wilhovsky SK, Goradia A, Hodgkiss-Harlow K, Hampton RY. HRD4/NPL4 is required for the proteasomal processing of ubiquitinated ER proteins. *Mol Biol Cell* 2001;12:4114–28.
- Ye Y, Meyer HH, Rapoport TA. The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. *Nature* 2001;414:652–6.
- Jarosch E, Taxis C, Volkwein C, et al. Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. *Nat Cell Biol* 2002;4:134–9.
- Braun S, Matuschewski K, Rape M, Thoms S, Jentsch S. Role of the ubiquitin-selective CDC48(UFD1/NPL4) chaperone (segregase) in ERAD of OLE1 and other substrates. *EMBO J* 2002;21:615–21.
- Elkabetz Y, Shapira I, Rabinovich E, Bar-Nun S. Distinct steps in dislocation of luminal endoplasmic reticulum-associated degradation substrates: roles of endoplasmic reticulum-bound p97/Cdc48p and proteasome. *J Biol Chem* 2004;279:3980–9.
- Davies JM, Tsuruta H, May AP, Weis WI. Conformational changes of p97 during nucleotide hydrolysis determined by small-angle X-Ray scattering. *Structure (Camb)* 2005;13:183–95.
- Rubin DM, Glickman MH, Larsen CN, Dhruvakumar S, Finley D. Active site mutants in the six regulatory particle ATPases reveal multiple roles for ATP in the proteasome. *EMBO J* 1998;17:4909–19.
- Kalies KU, Allan S, Sergeyenko T, Kroger H, Romisch K. The protein translocation channel binds proteasomes to the endoplasmic reticulum membrane. *EMBO J* 2005;24:2284–93.
- Verma R, Chen S, Feldman R, et al. Proteasomal proteomics: identification of nucleotide-sensitive proteasome-interacting proteins by mass spectrometric analysis of affinity-purified proteasomes. *Mol Biol Cell* 2000;11:3425–39.
- Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA. A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. *Nature* 2004;429:841–7.
- Schuberth C, Buchberger A. Membrane-bound Ubx2 recruits Cdc48 to ubiquitin ligases and their substrates to ensure efficient ER-associated protein degradation. *Nat Cell Biol* 2005;7:999–1006.
- Neuber O, Jarosch E, Volkwein C, Walter J, Sommer T. Ubx2 links the Cdc48 complex to ER-associated protein degradation. *Nat Cell Biol* 2005;7:993–8.
- Nita-Lazar M, Lennarz WJ. Pkc1p modifies CPY\* degradation in the ERAD pathway. *Biochem Biophys Res Commun* 2005;332:357–61.

---

**Correspondence:** Dr. S. Bar-Nun, Dept. of Biochemistry, Tel Aviv University, Ramat Aviv 69978, Israel.  
Phone: (972-3) 640-8984  
Fax: (972-3) 640-6834  
email: shoshbn@tauex.tau.ac.il