Protoplasma (1999) 207: 16–23

PROTOPLASMA
© Springer-Verlag 1999
Printed in Austria

Endoplasmic-reticulum-associated protein degradation inside and outside of the endoplasmic reticulum

N. Erwin Ivessa*, Claudia Kitzmüller, and **Maddalena de Virgilio**

Department of Molecular Genetics, University and Biocenter Vienna, Vienna

Received March 4, 1999
Accepted March 16, 1999

Summary. Newly synthesized polypeptides that enter the endomembrane system encounter a folding environment in the lumen of the endoplasmic reticulum (ER) constituted by enzymes, lectin-like proteins, and molecular chaperones. The folding process is under scrutiny of this abundant catalytic machinery, and failure of the new arrivals to assume a stable and functional conformation is met with targeting to proteolytic destruction, a process which has been termed ER-associated degradation (ERAD). In recent years it became clear that, in most cases, proteolysis appears to take place in the cytosol after retro-translocation of the substrate proteins from the ER, and to depend on the ubiquitin-proteasome pathway. On the other hand, proteolytic activities within the ER that have been widely neglected so far may also contribute to the turnover of proteins delivered to ERAD. Thus, ERAD is being deciphered as a complex process that requires communication-dependent regulated proteolytic activities within both the ER lumen and the cytosol. Here we discuss some recent findings on ERAD and their implications on possible mechanisms involved.

Keywords: Endoplasmic reticulum; Protease; Protein degradation; Retro-translocation; Ubiquitin-proteasome pathway.

Abbreviations: α1AT alpha-1-antitrypsin; apoB apolipoprotein B; BiP immunoglobulin-heavy-chain-binding protein; CFTR cystic fibrosis transmembrane conductance regulator; CPY carboxypeptidase Y; ER endoplasmic reticulum; ERAD ER-associated degradation; HMG-CoA 3-hydroxy-3-methylglutaryl coenzyme A; MHC major histocompatibility complex; PDI protein disulfide isomerase; TCR T cell antigen receptor.

Introduction

The rough domain of the endoplasmic reticulum (ER) is the entry point into the secretory pathway for most proteins that are destined for the endomembrane system (ER, Golgi apparatus, endosomes, lysosomes, secretory vesicles, and granules), for the plasma membrane, and for secretion. In higher eukaryotes, these proteins, synthesized on membrane-bound polysomes, are co-translationally inserted into the ER by passage through the translocation channel comprised of the Sec61 protein complex (Rapoport et al. 1996). This implies that the nascent polypeptide chain is transferred into the ER lumen in an extended and thus essentially completely unfolded state and requires folding processes to take place inside the ER, allowing the protein to attain its native conformation. In addition, many proteins undergo co- and posttranslational modifications early on during their biogenesis. These include N-linked glycosylation, and, owing to the oxidizing environment of the ER lumen, the formation of disulfide bonds. The folding process per se is facilitated by the presence of high concentrations of molecular chaperones, such as BiP (also called GRP78), GRP94, and PDI, as well as of lectinlike molecules, such as calnexin and calreticulin (reviewed by Leitzgen and Haas 1998; and see below). Folding is intended to result in a rather stable conformation of the newly synthesized protein, enabling it to assume a functional role, either within the endomembrane system or after secretion. Failure of the protein to comply with the rules of folding apparently results in its removal by proteolytic degradation, a process which takes place at or close to the site of synthesis at the ER and, therefore, has been called ER-associated degradation (ERAD).

* Correspondence and reprints: Department of Molecular Genetics, Biocenter Vienna, Dr.-Bohr-Gasse 9/2, A-1030 Vienna, Austria.

Common features of ERAD for different substrate proteins

Already from early studies on the subject it became apparent that ERAD is a process independent of lysosomal degradation and autophagy (Klausner and Sitia 1990). It involves initial retention of the substrate protein in the ER, which is followed, sometimes after a considerable lag phase, by rapid degradation without detectable proteolytic intermediates. Notably, the substrates for ERAD may be transmembrane or luminally disposed proteins and are mutant, truncated, or otherwise misfolded proteins. Subunits of oligomeric protein complexes synthesized in excess of the stoichiometrically required amount (e.g., TCR subunits), and even wild-type proteins (CFTR, apoB, and HMG-CoA reductase), some of which are metabolically regulated, may also be subject to ERAD (Table 1).

As a general observation, it turned out in recent years that ERAD is, in fact, a cytosolic process mediated by the 26S proteasome, after extraction of the substrate protein from the ER (retro-translocation; Sommer and Wolf 1997). Arriving in the ER lumen, the newly synthesized protein encounters a variety of lectinlike proteins and chaperones, a selected set of which it may transiently associate with, resulting in initial retention and allowing folding processes to occur during a limited period of time (see below). After a protein has failed the folding endeavors of the cellular machinery, a recognition event must occur, followed by targeting of the protein to the degradative pathway. This process should imply retargeting of the misfolded protein to the translocon on the luminal side of the ER and reprogramming of the translocon for retro-translocation (Sommer and Wolf 1997). Alternatively, but less likely, in a reversal of protein import, the nascent polypeptide may simply be extracted from the translocation channel, if it remained directly or indirectly associated with the translocon during the folding, recognition, and targeting process.

In many cases the addition of ubiquitin moieties to the substrate protein, a reaction that takes place in the cytosol, has been observed (Ward et al. 1995, Jensen et al. 1995; for a review, see Bonifacino and Weissman 1998) (Table 1). In yeast this process is mediated by two ubiquitin-conjugating enzymes, Ubc6p, an ER transmembrane protein with its functional domain facing the cytosol, and soluble Ubc7p, which is recruited to the ER membrane by Cue1p (Biederer et al. 1997). Novel proteins, such as Der1p, Der3p/Hrd1p, and Hrd3p, that may be involved in targeting to ERAD and retro-translocation and whose precise function needs to be established, have been identified in yeast (see Sommer and Wolf 1997). Furthermore, it has been proposed that ubiquitination (Bordallo et al. 1998, de Virgilio et al. 1998), or the activity of proteasomes (Mayer et al. 1998, Plemper et al. 1998) are required for retro-translocation to occur. Glycoproteins are usually deglycosylated during or after retro-translocation by a cytosolic N-glycanase (Wiertz et al. 1996), although considerable processing of glycans within the ER lumen has also been demonstrated (Le et al. 1992, Weng and Spiro 1997, de Virgilio et al. 1998).

Role of ER-resident and cytosolic chaperones in ERAD

The lectinlike proteins calnexin and calreticulin were found to bind to a number of newly synthesized glycoproteins, where they function as a retention device, allowing the substrate proteins to profit from the folding environment of the ER (Trombetta and Helenius 1998). Correctly folded proteins appear to be released from calnexin and calreticulin as soon as their folding process is completed. In the case of aberrant proteins, the final dissociation from the lectinlike proteins and targeting to the proteolytic pathway requires a specific oligosaccharide structure which is acquired by trimming of mannose residues from the N-linked glycan (Jakob et al. 1998, M. Yang et al. 1998). Among the proteins that bind to calnexin and/or calreticulin are CFTR, MHC class I heavy chain, and apoB (Table 1).

A prominent chaperone of the ER is BiP, which recognizes hydrophobic and exposed patches that would be buried in the correctly folded protein. BiP may serve multiple functions, such as limiting the accessibility of the translocation channel on the luminal side in the absence of ongoing translation and translocation, retention of proteins in the ER, promoting their folding process, and possibly delivery of misfolded proteins to ERAD (Leitzgen and Haas 1998). Interestingly, the half time of interaction of BiP with the immunoglobulin light chain, a substrate protein for ERAD, correlates precisely with the half-life of the immunoglobulin subunit and thus depends on the physical stability of the complex formed between BiP and an unfolded region of the substrate protein (Skowronek et al. 1998). Further interaction

Table 1. Features of ERAD for some known substrate proteins

Substrate protein	Reason(s) for ERAD	Chaperone(s) involved	Ubiquitination	References
Alpha-1-antitrypsin	point mutation (PiZ), misfolding, ER aggregation	calnexin, UDP-glucose:glycoprotein glucosyltransferase, BiP (in yeast)	yes[a]	Le et al. 1992, 1994; Lomas et al. 1992; Qu et al. 1996; Teckman and Perlmutter 1996; Werner et al. 1996; Choudhury et al. 1997; Brodsky et al. 1999
ApoB	lack of assembly into a lipoprotein particle due to low lipid availability	BiP, GRP94, ERp60, ERp72, calreticulin, calnexin, Hsp70	yes	Zhou et al. 1995, 1998; Yeung et al. 1996; Fisher et al. 1997; Adeli et al. 1997; Wu et al. 1997; Chen et al. 1998; Linnik and Herscovitz 1998
CFTR deltaF508	point mutation (deltaF508), misfolding, cytoplasmic aggregation ("aggresomes")	calnexin, Hsp70, Hsp90	yes	Y. Yang et al. 1993; Pind et al. 1994; Jensen et al. 1995; Ward et al. 1995; M. Loo et al. 1998; Sato et al. 1998
Mutant CPY (yeast)	point mutation, misfolding	BiP	yes	Hiller et al. 1996; Plemper et al. 1997; Bordallo et al. 1998
HMG-CoA reductase (mammalian)	regulated by mevalonate	?[b]	nd[c]	McGee et al. 1996
HMG-CoA reductase (yeast)	regulated by mevalonate	?	yes	Hampton and Bhakta 1997
MHC class I heavy chain	lack of assembly, induced by human cytomegalovirus proteins US2 and US11	BiP, ERp60, calreticulin, calnexin, tapasin	nd[d] yes[e]	Wiertz et al. 1996; Ortmann et al. 1997; Pamer and Cresswell 1998; Lindquist et al. 1998; Hughes et al. 1998; Morrice et al. 1998
Mutant Pdr5p (yeast)	point mutation, misfolding	?	yes	Plemper et al. 1998
P-glycoprotein (human)	point mutation, misfolding	calnexin	nd	Jensen et al. 1995; T. Loo and Clarke 1994, 1998
Mutant pro-alpha factor (yeast)	mutation (lack of glycosylation), misfolding	BiP, calnexin	nd	McCracken and Brodsky 1996; Brodsky et al. 1999
Mutant Sec61p (yeast)	mutation, misfolding	?	yes	Sommer and Jentsch 1993; Biederer et al. 1996
Mutant Ste6p (yeast)	mutation, misfolding	?	yes	Loayza et al. 1998
TCR alpha	failure to assemble, charged amino acids in the transmembrane domain	BiP, calreticulin, calnexin	no[f] yes[g]	Suzuki et al. 1991; van Leeuwen and Kearse 1996, 1997; Huppa and Ploegh 1997; Yu et al. 1997; Y. Yang et al. 1998
TCR delta	failure to assemble, charged amino acids in the transmembrane domain	calreticulin, calnexin	yes	van Leeuwen and Kearse 1996, 1997; Y. Yang et al. 1998
Truncated ribophorin I RI_{332}	failure to assemble	calnexin	yes	de Virgilio et al. 1998
Mutant uracil permease (yeast)	mutation, misfolding	?	yes	Galan et al. 1998

[a] Qu et al. (1996) reported on the ubiquitination of calnexin found in association with α1AT
[b] So far none reported
[c] Not detected
[d] Wiertz et al. 1996
[e] Hughes et al. 1997
[f] Yu et al. (1997) found that a TCR alpha variant having all lysine residues replaced by arginines is also a substrate for proteasomal degradation that probably is ubiquitin-independent
[g] Yang et al. 1998

partners of BiP include apoB and mutant carboxypeptidase Y (CPY) in yeast (Table 1).

Other ER-resident chaperones that have been found to interact with substrate proteins for ERAD are GRP94, ERp60, and ERp72 (Table 1 and below). The role of PDI as a chaperone in ERAD is less clear thus far, as its binding to substrate proteins may be due to its disulfide isomerase activity. In addition, some specialized chaperones have been identified that appear to interact only with certain substrate proteins. These include tapasin for MHC class I heavy chain (Ortmann et al. 1997) and receptor-associated protein (RAP) for proteins belonging to the low-density lipoprotein receptor family (Bu and Schwartz 1998, Willnow 1998).

Hsp70, an abundant cytosolic chaperone, has been detected to interact with proteins that are delivered to degradation, most notably with transmembrane proteins, such as CFTR, whose cytoplasmic parts remain exposed (Y. Yang et al. 1993). ApoB that is inefficiently translocated into the lumen of the ER, especially under conditions of low lipid availability, was also shown to bind to Hsp70 cotranslationally (Zhou et al. 1995, 1998). Furthermore, recent evidence indicates that also cytosolic Hsp90 associates with CFTR and that degradation of the protein is accelerated when the interaction with the chaperone is pharmacologically perturbed (M. Loo et al. 1998).

Aggregation of substrate proteins

One of the cellular reactions to the expression of proteins that cannot fold correctly is aggregation. In the context of ERAD this has been observed in a variety of different manifestations, mostly depending on the substrate protein. For instance, the PiZ variant of alpha-1-antitrypsin (α1AT) is known to form insoluble oligo- and multimeric aggregates within the lumen of the ER, whereas only part of the protein appears to be degraded (Le et al. 1992, Lomas et al. 1992). Aggregate formation correlates with the severe α1AT deficiency phenotype associated with liver disease (Teckman and Perlmutter 1996). Another ER storage phenotype is the formation of so-called Russell bodies, where immunoglobulins consisting of mutant IgM heavy chains assembled with light chains accumulate in dilated ER cisternae and cannot be secreted or degraded (Valetti et al. 1991).

A novel type of aggregate, formed by cytosolic accumulation of CFTR and other transmembrane proteins that fold inefficiently in the ER, was recently discovered and called "aggresome" (Johnston et al. 1998). These structures are characterized as stable, high-molecular-weight, detergent-insoluble, multiubiquitinated aggregates. Their formation was detected after the inhibition of proteasomes, or after overexpression of the wild-type or mutated substrate proteins resulting in saturation of proteasome activity. Intact microtubules are required for the localization of the aggregates close to the microtubule-organizing center, but not for their biogenesis itself. Furthermore, it was shown that the intermediate-filament protein vimentin redistributes upon formation of aggresomes, forming a cagelike structure around the aggregated proteins. It was proposed that the appearance of aggresomes is a general cellular response to overexpression of proteins, when the capacity of intracellular folding and degradation machineries is exceeded (Johnston et al. 1998).

Protein processing and degradation within the ER?

It was originally believed that ERAD takes place within the ER lumen or a closely related tubular-vesicular compartment. This assumption was soon generally dismissed after the involvement of the ubiquitin-proteasome pathway became clear. However, a number of observations, even from recent origin, seems difficult to reconcile with exclusively cytosolic degradation, and several findings point towards the ER lumen or even the ER membrane as a site of proteolytic processing. Two PDI-like proteins, ERp60 and ERp72, with cysteine protease activity were found to process proteolytically and degrade resident ER proteins in vitro (Urade et al. 1993). Moreover, ERp60, also called ER-60 protease or ERp57, was shown to bind to and degrade human lysozyme overexpressed in mouse fibroblasts and in vitro, specifically when the protein had been mutated and is misfolded or denatured (Otsu et al. 1995); similarly, association of PDI with misfolded lysozyme variants was observed (Otsu et al. 1994). In addition, ERp60 was also identified as a component of the ER quality control system, as it binds transiently to newly synthesized glycoproteins and appears to act in concert with calnexin and calreticulin (Oliver et al. 1997). In this case, the interaction of ERp60 with its target protein depends on glucose trimming of the N-linked glycan. An involvement of ERp60 in the assembly of MHC class I molecules has been described (Lindquist et al. 1998,

Hughes et al. 1998, Morrice et al. 1998). The association of ERp60 and other ER proteins, such as BiP, with apoB in human hepatoma cells was also detected; in fact, ERp60 apparently causes the generation of distinct proteolytic fragments from the apolipoprotein (Adeli et al. 1997). The appearance of distinct apoB fragments has been ascribed to a dithiothreitol-sensitive, but cysteine-protease-inhibitor- and proteasome-inhibitor-insensitive protease in the ER lumen (Wu et al. 1997). Interestingly, the role of ERp60 in protein maturation within the lumen of the ER seems to vary according to the context; thus the protein may fulfill the function of a chaperone and/or a protease.

Recently, a luminally disposed proteolytic activity, whose precise nature remains to be elucidated, was detected that acts on certain mutated forms of human P-glycoprotein (P-gp), a membrane protein of the ATP-binding cassette protein family with 12 transmembrane domains, and results in the appearance of an unstable proteolytically processed product (T. Loo and Clarke 1998). After removal of a protease-sensitive site within the first exoplasmic loop of P-gp, the protein remained uncleaved and stable.

It is well established that the steady-state levels of HMG-CoA reductase are metabolically regulated by ERAD (among other mechanisms), and that this regulation depends on the N-terminal membrane domain of the protein. Although HMG-CoA reductase appears to be degraded by the proteasome in vivo (McGee et al. 1996, Hampton et al. 1996), distinct cleavage products have been observed in vitro that are generated due to the activity of (a) membrane-associated cysteine protease(s) by processing the substrate within the membrane domain (Moriyama et al. 1998).

One of the best-characterized proteolytic enzymes in the ER is the signal peptidase complex, which cleaves short peptide segments from newly synthesized polypeptides required for their targeting to and cotranslational insertion into the ER. It has been proposed that, in addition to the removal of the signal peptide, the enzyme may also be responsible for the proteolytic processing of the H2a subunit of the asialoglycoprotein receptor at the luminal end of the membrane span, giving rise to a fragment comprising the ectodomain, which is secreted, and a membrane-bound fragment, which is delivered to ERAD (Tolchinsky et al. 1996). Moreover, a link between signal peptidase activity and the degradation of aberrant proteins has been observed in yeast, as the proteolytic processing and the degradation of two chimeric membrane proteins that lack N-terminal signal peptides were severely inhibited, when the cells were carrying a mutation in the Sec11p subunit (Mullins et al. 1995); this subunit is essential for cell growth and signal peptide cleavage (Bohni et al. 1988). Thus, it appears that signal peptidase may, at least under certain circumstances, such as in the presence of a signal/anchor sequence, be involved in quality control processes in the ER.

Results obtained from experiments with permeabilized cells and with in vitro reconstituted systems did not firmly establish, nor disprove, the ER as a site of protein degradation. An early study using streptolysin O-permeabilized fibroblasts expressing TCR alpha and beta chain chimeras and isolated microsomes from these cells demonstrated that neither intracellular membrane transport nor ATP or cytosol are required for the proteolytic breakdown of these proteins (Stafford and Bonifacino 1991). Similar findings were reported on the degradation of the H2a subunit of the human asialoglycoprotein receptor in semi-permeabilized CHO cells (Wikstrom and Lodish 1992). More recently, however, in vitro systems that reproduce ERAD in yeast were developed, and requirements for cytosol, calnexin, and ATP were inferred for the degradation of substrate glycoproteins (McCracken and Brodsky 1996, Werner et al. 1996).

From these and other data, the picture emerges that proteolytic activities within the lumen of the ER may be tightly linked to the quality control apparatus of the ER and thus contribute to ERAD, possibly by making initial cleavages into polypeptides targeted for degradation. This may be required for efficient extraction of luminal and polytopic membrane proteins from the ER for degradation by the proteasome in the cytosol.

Perspective

The current knowledge on ERAD reflects the view of a multifaceted and highly regulated process with a wide variety of players, only some of which may be involved in the degradation of a specific substrate. Retention in the ER and degradation are clearly separable and governed by differing mechanisms. Diminishing ER degradation usually does not result in improved transport from the ER (see Kowalski et al. 1998), but promoting assembly, e.g., in the case of apoB, does (Sakata et al. 1993). This has to be seen in

the light of a concept, in which intracellular transport processes rely on positive sorting signals and their interactions with soluble cargo adaptors and coat proteins of vesicular carriers (Kuehn et al. 1998).

The challenge of the next years will be to integrate the rudimentary mosaic picture obtained so far mostly by degradation analyses of many different substrate proteins into a coherent model of ERAD in which specific functions are assigned to all the major contributing factors. One focus will be on trying to understand the coordinated regulation between two topologically distinct compartments, the ER lumen and the cytosol, in the context of ERAD. Another important point will be to elucidate the actual targeting step at which the system makes the decision to give up further folding attempts and to deliver the polypeptide to the degradative machinery. Clearly, this will require the extensive characterization of some of the proteins known to participate in ERAD, and possibly the identification of novel components, e.g., by using yeast genetics.

Acknowledgements

Work from the authors' laboratory has been supported by grants from the Austrian Science Foundation (FWF Proj. P12562-MOB), the Austrian National Bank (OeNB Proj. 6314) and the Kamillo Eisner Foundation. We sincerely wish to apologize to all those colleagues whose work, though relevant for the topic of the review, could not be cited due to space limitations.

References

Adeli K, Macri J, Mohammadi A, Kito M, Urade R, Cavallo D (1997) Apolipoprotein B is intracellularly associated with an ER-60 protease homologue in HepG2 cells. J Biol Chem 272: 22489–22494

Biederer T, Volkwein C, Sommer T (1996) Degradation of subunits of the Sec61p complex, an integral component of the ER membrane, by the ubiquitin-proteasome pathway. EMBO J 15: 2069–2076

– – – (1997) Role of Cue1p in ubiquitination and degradation at the ER surface. Science 278: 1806–1809

Bohni PC, Deshaies RJ, Schekman RW (1988) SEC11 is required for signal peptide processing and yeast cell growth. J Cell Biol 106: 1035–1042

Bonifacino JS, Weissman AM (1998) Ubiquitin and the control of protein fate in the secretory and endocytic pathways. Annu Rev Cell Dev Biol 14: 19–57

Bordallo J, Plemper RK, Finger A, Wolf DH (1998) Der3p/Hrd1p is required for endoplasmic reticulum-associated degradation of misfolded lumenal and integral membrane proteins. Mol Biol Cell 9: 209–222

Brodsky JL, Werner ED, Dubas ME, Goeckeler JL, Kruse KB, McCracken AA (1999) The requirement for molecular chaperones during endoplasmic reticulum-associated protein degradation demonstrates that protein export and import are mechanistically distinct. J Biol Chem 274: 3453–3460

Bu G, Schwartz AL (1998) RAP, a novel type of ER chaperone. Trends Cell Biol 8: 272–276

Chen Y, Le Caherec F, Chuck SL (1998) Calnexin and other factors that alter translocation affect the rapid binding of ubiquitin to apoB in the Sec61 complex. J Biol Chem 273: 11887–11894

Choudhury P, Liu Y, Bick RJ, Sifers RN (1997) Intracellular association between UDP-glucose:glycoprotein glucosyltransferase and an incompletely folded variant of alpha1-antitrypsin. J Biol Chem 272: 13446–13451

de Virgilio M, Weninger H, Ivessa NE (1998) Ubiquitination is required for the retro-translocation of a short-lived luminal endoplasmic reticulum glycoprotein to the cytosol for degradation by the proteasome. J Biol Chem 273: 9734–9743

Fisher EA, Zhou M, Mitchell DM, Wu X, Omura S, Wang H, Goldberg AL, Ginsberg HN (1997) The degradation of apolipoprotein B100 is mediated by the ubiquitin-proteasome pathway and involves heat shock protein 70. J Biol Chem 272: 20427–20434

Galan JM, Cantegrit B, Garnier C, Namy O, Haguenauer-Tsapis R (1998) "ER degradation" of a mutant yeast plasma membrane protein by the ubiquitin-proteasome pathway. FASEB J 12: 315–323

Hampton RY, Bhakta H (1997) Ubiquitin-mediated regulation of 3-hydroxy-3-methylglutaryl-CoA reductase. Proc Natl Acad Sci USA 94: 12944–12948

– Gardner RG, Rine J (1996) Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. Mol Biol Cell 7: 2029–2044

Hiller MM, Finger A, Schweiger M, Wolf DH (1996) ER degradation of a misfolded luminal protein by the cytosolic ubiquitin-proteasome pathway. Science 273: 1725–1728

Hughes EA, Cresswell P (1998) The thiol oxidoreductase ERp57 is a component of the MHC class I peptide-loading complex. Curr Biol 8: 709–712

– Hammond C, Cresswell P (1997) Misfolded major histocompatibility complex class I heavy chains are translocated into the cytoplasm and degraded by the proteasome. Proc Natl Acad Sci USA 94: 1896–1901

Huppa JB, Ploegh HL (1997) The alpha chain of the T cell antigen receptor is degraded in the cytosol. Immunity 7: 113–122

Jakob CA, Burda P, Roth J, Aebi M (1998) Degradation of misfolded endoplasmic reticulum glycoproteins in *Saccharomyces cerevisiae* is determined by a specific oligosaccharide structure. J Cell Biol 142: 1223–1233

Jensen TJ, Loo MA, Pind S, Williams DB, Goldberg AL, Riordan JR (1995) Multiple proteolytic systems, including the proteasome, contribute to CFTR processing. Cell 83: 129–135

Johnston JA, Ward CL, Kopito RR (1998) Aggresomes: a cellular response to misfolded proteins. J Cell Biol 143: 1883–1898

Klausner RD, Sitia R (1990) Protein degradation in the endoplasmic reticulum. Cell 62: 611–614

Kowalski JM, Parekh RN, Mao J, Wittrup KD (1998) Protein folding stability can determine the efficiency of escape from endoplasmic reticulum quality control. J Biol Chem 273: 19453–19458

Kuehn MJ, Herrmann JM, Schekman R (1998) COPII-cargo interactions direct protein sorting into ER-derived transport vesicles. Nature 391: 187–190

Le A, Ferrell GA, Dishon DS, Le QQ, Sifers RN (1992) Soluble aggregates of the human PiZ alpha 1-antitrypsin variant are degraded within the endoplasmic reticulum by a mechanism sensitive to inhibitors of protein synthesis. J Biol Chem 267: 1072–1080

Le A, Steiner JL, Ferrell GA, Shaker JC, Sifers RN (1994) Association between calnexin and a secretion-incompetent variant of human alpha 1-antitrypsin. J Biol Chem 269: 7514–7519

Leitzgen K, Haas IG (1998) Protein maturation in the endoplasmic reticulum. Chemtracts Biochem Mol Biol 11: 423–445

Lindquist JA, Jensen ON, Mann M, Hammerling GJ (1998) ER-60, a chaperone with thiol-dependent reductase activity involved in MHC class I assembly. EMBO J 17: 2186–2195

Linnik KM, Herscovitz H (1998) Multiple molecular chaperones interact with apolipoprotein B during its maturation: the network of endoplasmic reticulum-resident chaperones (ERp72, GRP94, calreticulin, and BiP) interacts with apolipoprotein B regardless of its lipidation state. J Biol Chem 273: 21368–21373

Loayza D, Tam A, Schmidt WK, Michaelis S (1998) Ste6p mutants defective in exit from the endoplasmic reticulum (ER) reveal aspects of an ER quality control pathway in *Saccharomyces cerevisiae*. Mol Biol Cell 9: 2767–2784

Lomas DA, Evans DL, Finch JT, Carrell RW (1992) The mechanism of Z alpha 1-antitrypsin accumulation in the liver. Nature 357: 605–607

Loo MA, Jensen TJ, Cui L, Hou YX, Chang XB, Riordan JR (1998) Perturbation of hsp90 interaction with nascent CFTR prevents its maturation and accelerates its degradation by the proteasome. EMBO J 17: 6879–6887

Loo TW, Clarke DM (1994) Prolonged association of temperature-sensitive mutants of human P-glycoprotein with calnexin during biogenesis. J Biol Chem 269: 28683–28689

– – (1998) Superfolding of the partially unfolded core-glycosylated intermediate of human P-glycoprotein into the mature enzyme is promoted by substrate-induced transmembrane domain interactions. J Biol Chem 273: 14671–14674

Mayer TU, Braun T, Jentsch S (1998) Role of the proteasome in membrane extraction of a short-lived ER-transmembrane protein. EMBO J 17: 3251–3257

McCracken AA, Brodsky JL (1996) Assembly of ER-associated protein degradation in vitro: dependence on cytosol, calnexin, and ATP. J Cell Biol 132: 291–298

McGee TP, Cheng HH, Kumagai H, Omura S, Simoni RD (1996) Degradation of 3-hydroxy-3-methylglutaryl-CoA reductase in endoplasmic reticulum membranes is accelerated as a result of increased susceptibility to proteolysis. J Biol Chem 271: 25630–25638

Moriyama T, Sather SK, McGee TP, Simoni RD (1998) Degradation of HMG-CoA reductase in vitro: cleavage in the membrane domain by a membrane-bound cysteine protease. J Biol Chem 273: 22037–22043

Morrice NA, Powis SJ (1998) A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules. Curr Biol 8: 713–716

Mullins C, Lu Y, Campbell A, Fang H, Green N (1995) A mutation affecting signal peptidase inhibits degradation of an abnormal membrane protein in Saccharomyces cerevisiae. J Biol Chem 270: 17139–17147

Oliver JD, van der Wal FJ, Bulleid NJ, High S (1997) Interaction of the thiol-dependent reductase ERp57 with nascent glycoproteins. Science 275: 86–88

Ortmann B, Copeman J, Lehner PJ, Sadasivan B, Herberg JA, Grandea AG, Riddell SR, Tampe R, Spies T, Trowsdale J, Cresswell P (1997) A critical role for tapasin in the assembly and function of multimeric MHC class I-TAP complexes. Science 277: 1306–1309

Otsu M, Omura F, Yoshimori T, Kikuchi M (1994) Protein disulfide isomerase associates with misfolded human lysozyme in vivo. J Biol Chem 269: 6874–6877

– Urade R, Kito M, Omura F, Kikuchi M (1995) A possible role of ER-60 protease in the degradation of misfolded proteins in the endoplasmic reticulum. J Biol Chem 270: 14958–14961

Pamer E, Cresswell P (1998) Mechanisms of MHC class I – restricted antigen processing. Annu Rev Immunol 16: 323–358

Pind S, Riordan JR, Williams DB (1994) Participation of the endoplasmic reticulum chaperone calnexin (p88, IP90) in the biogenesis of the cystic fibrosis transmembrane conductance regulator. J Biol Chem 269: 12784–12788

Plemper RK, Bohmler S, Bordallo J, Sommer T, Wolf DH (1997) Mutant analysis links the translocon and BiP to retrograde protein transport for ER degradation. Nature 388: 891–895

– Egner R, Kuchler K, Wolf DH (1998) Endoplasmic reticulum degradation of a mutated ATP-binding cassette transporter Pdr5 proceeds in a concerted action of Sec61 and the proteasome. J Biol Chem 273: 32848–32856

Qu D, Teckman JH, Omura S, Perlmutter DH (1996) Degradation of a mutant secretory protein, alpha1-antitrypsin Z, in the endoplasmic reticulum requires proteasome activity. J Biol Chem 271: 22791–22795

Rapoport TA, Jungnickel B, Kutay U (1996) Protein transport across the eukaryotic endoplasmic reticulum and bacterial inner membranes. Annu Rev Biochem 65: 271–303

Sakata N, Wu X, Dixon JL, Ginsberg HN (1993) Proteolysis and lipid-facilitated translocation are distinct but competitive processes that regulate secretion of apolipoprotein B in Hep G2 cells. J Biol Chem 268: 22967–22970

Sato S, Ward CL, Kopito RR (1998) Cotranslational ubiquitination of cystic fibrosis transmembrane conductance regulator in vitro. J Biol Chem 273: 7189–7192

Skowronek MH, Hendershot LM, Haas IG (1998) The variable domain of nonassembled Ig light chains determines both their half-life and binding to the chaperone BiP. Proc Natl Acad Sci USA 95: 1574–1578

Sommer T, Jentsch S (1993) A protein translocation defect linked to ubiquitin conjugation at the endoplasmic reticulum. Nature 365: 176–179

– Wolf DH (1997) Endoplasmic reticulum degradation: reverse protein flow of no return. FASEB J 11: 1227–1233

Stafford FJ, Bonifacino JS (1991) A permeabilized cell system identifies the endoplasmic reticulum as a site of protein degradation. J Cell Biol 115: 1225–1236

Suzuki CK, Bonifacino JS, Lin AY, Davis MM, Klausner RD (1991) Regulating the retention of T-cell receptor alpha chain variants within the endoplasmic reticulum: Ca(2+)-dependent association with BiP. J Cell Biol 114: 189–205

Teckman JH, Perlmutter DH (1996) The endoplasmic reticulum degradation pathway for mutant secretory proteins alpha1-antitrypsin Z and S is distinct from that for an unassembled membrane protein. J Biol Chem 271: 13215–13220

Tolchinsky S, Yuk MH, Ayalon M, Lodish HF, Lederkremer GZ (1996) Membrane-bound versus secreted forms of human asialoglycoprotein receptor subunits: role of a juxtamembrane pentapeptide. J Biol Chem 271: 14496–14503

Trombetta ES, Helenius A (1998) Lectins as chaperones in glycoprotein folding. Curr Opin Struct Biol 8: 587–592

Urade R, Takenaka Y, Kito M (1993) Protein degradation by ERp72 from rat and mouse liver endoplasmic reticulum. J Biol Chem 268: 22004–22009

Valetti C, Grossi CE, Milstein C, Sitia R (1991) Russell bodies: a general response of secretory cells to synthesis of a mutant immunoglobulin which can neither exit from, nor be degraded in, the endoplasmic reticulum. J Cell Biol 115: 983–994

Van Leeuwen JEM, Kearse KP (1996) The related molecular chaperones calnexin and calreticulin differentially associate with nascent T cell antigen receptor proteins within the endoplasmic reticulum. J Biol Chem 271: 25345–25349

– – (1997) Reglucosylation of N-linked glycans is critical for calnexin assembly with T cell receptor (TCR) alpha proteins but not TCR beta proteins. J Biol Chem 272: 4179–4186

Ward CL, Omura S, Kopito RR (1995) Degradation of CFTR by the ubiquitin-proteasome pathway. Cell 83: 121–127

Weng S, Spiro RG (1997) Demonstration of a peptide:N-glycosidase in the endoplasmic reticulum of rat liver. Biochem J 322: 655–661

Werner ED, Brodsky JL, McCracken AA (1996) Proteasome-dependent endoplasmic reticulum-associated protein degradation: an unconventional route to a familiar fate. Proc Natl Acad Sci USA 93: 13797–13801

Wiertz EJ, Tortorella D, Bogyo M, Yu J, Mothes W, Jones TR, Rapoport TA, Ploegh HL (1996) Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 384: 432–438

Wikstrom L, Lodish HF (1992) Endoplasmic reticulum degradation of a subunit of the asialoglycoprotein receptor in vitro: vesicular transport from endoplasmic reticulum is unnecessary. J Biol Chem 267: 5–8

Willnow TE (1998) Receptor-associated protein (RAP): a specialized chaperone for endocytic receptors. Biol Chem 379: 1025–1031

Wu X, Sakata N, Lele KM, Zhou M, Jiang H, Ginsberg HN (1997) A two-site model for ApoB degradation in HepG2 cells. J Biol Chem 272: 11575–11580

Yang M, Omura S, Bonifacino JS, Weissman AM (1998) Novel aspects of degradation of T cell receptor subunits from the endoplasmic reticulum (ER) in T cells: importance of oligosaccharide processing, ubiquitination, and proteasome-dependent removal from ER membranes. J Exp Med 187: 835–846

Yang Y, Janich S, Cohn JA, Wilson JM (1993) The common variant of cystic fibrosis transmembrane conductance regulator is recognized by hsp70 and degraded in a pre-Golgi nonlysosomal compartment. Proc Natl Acad Sci USA 90: 9480–9484

Yeung SJ, Chen SH, Chan L (1996) Ubiquitin-proteasome pathway mediates intracellular degradation of apolipoprotein B. Biochemistry 35: 13843–13848

Yu H, Kaung G, Kobayashi S, Kopito RR (1997) Cytosolic degradation of T-cell receptor alpha chains by the proteasome. J Biol Chem 272: 20800–20804

Zhou M, Wu X, Huang LS, Ginsberg HN (1995) Apoprotein B100, an inefficiently translocated secretory protein, is bound to the cytosolic chaperone, heat shock protein 70. J Biol Chem 270: 25220–25224

– Fisher EA, Ginsberg HN (1998) Regulated co-translational ubiquitination of apolipoprotein B100: a new paradigm for proteasomal degradation of a secretory protein. J Biol Chem 273: 24649–24653