Plant Cell Monogr (4)
D.G. Robinson: The Plant Endoplasmic Reticulum
DOI 10.1007/7089_066/Published online: 23 March 2006

Endoplasmic Reticulum-associated Protein Degradation in Plant Cells

Aldo Ceriotti[1] (✉) · Lynne M. Roberts[2]

[1]Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Via Bassini 15, 20133 Milan, Italy
ceriotti@ibba.cnr.it

[2]Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

Abstract The endoplasmic reticulum (ER) is equipped with a quality control function that retains misfolded and unassembled proteins and allows only structurally mature polypeptides to be transported to their final destination. The retained proteins are eventually retro-translocated to the cytosol and destroyed by a process called endoplasmic reticulum-associated degradation (ERAD). Besides being involved in the degradation of aberrant polypeptides, the ERAD pathway is also used to regulate cellular functions and is exploited by some plant and bacterial toxins to reach the cytosol after internalization by target cells. After summarizing the general characteristics of the ERAD pathway, we describe the features of known plant ERAD substrates and of the plant degradative machinery, highlighting the role of protein disposal in the response to ER stress.

1 Introduction

Protein folding is an error-prone process, and while certain polypeptides can fold quite efficiently, a relevant fraction of other proteins fails to reach a native conformation even under normal conditions. These misfolded polypeptides may be endowed with new and deleterious activities, act as dominant negative mutants, or may more generally get involved in spurious interactions that interfere with normal cellular functions. As such, they must be rapidly eliminated. Indeed, when the overall efficiency of protein biogenesis in a mammalian cell population was measured, it was found that up to two-thirds of newly synthesized polypeptides were rapidly destroyed (Schubert et al. 2000). In addition, cellular proteins are subjected to post-synthetic damage due to the action of reactive small molecules, proteases, and to changes in primary sequence (protein ageing). These post-synthetic changes, as well as exposure to high temperatures, can lead to a degree of protein unfolding that can be deleterious to the cell. It is therefore clear that cells must have a way to deal with this continually generated mass of aberrant and potentially damaging protein. The way the endoplasmic reticulum (ER) copes with the problem of disposing of misfolded proteins is the subject of this chapter.

2
Retention in the ER as a Prelude to Degradation

Many of the proteins that are inserted into the ER lumen or into the ER membrane are not residents of this organelle and so must be transported to other intracellular sites, or be secreted. About 20 years ago, it became apparent that proteins entering the ER are subjected to a quality-control mechanism that allows only correctly folded and (when applicable) assembled polypeptides to be transported to their final destination (reviewed in Ellgaard and Helenius 2003). It was later realized that rather than being degraded within the lumen of the ER, soluble and membrane bound secretory proteins that fail to fold are destroyed after being dislocated to the cytosol, where they become substrates of proteasomes. Thus, although the vacuole can also contribute to the disposal of ER and secretory proteins (Hong et al. 1996; Tamura et al. 2004), retention and proteasomal degradation appears to be a common mechanism that cells use to avoid the potentially dangerous expression of aberrant polypeptides. This disposal pathway is often referred to as ER-associated protein degradation (ERAD) and considerable progress has been made during the last decade towards understanding its individual steps at the molecular level.

Different mechanisms can contribute to the retention of misfolded proteins in the ER. One of them could be the absence of an exposed export signal. According to current models, export of proteins from the ER can occur by bulk-flow or can be mediated by receptors that recognize specific signals on the exported proteins (see chapt. by Aniento et al., this volume). If these signals are not exposed on the surface of the molecule because of misfolding, the protein will be retained in the folding compartment. The retention of unfolded proteins in the ER can also be due to their association with molecular chaperones, which in turn are maintained in the ER at steady state because they bear signals for retrieval from downstream compartments or because they form an insoluble matrix and are therefore excluded from budding vesicles.

A role for protein trafficking in the ERAD pathway is suggested by the finding that a generalized block in the transport of proteins to the Golgi complex can inhibit the degradation of certain substrates (reviewed in Ahner and Brodsky 2004). In addition, it has been shown that certain misfolded proteins can leave the ER in transport vesicles only to be returned prior to degradation (Vashist et al. 2001; Yamamoto et al. 2001; Sato et al. 2004). However, since a block in protein transport can also have a direct impact on ER structure and homeostasis, it remains to be established whether certain proteins must recycle from the Golgi to be degraded, or whether the observed effects are due to a general perturbation of ER functions.

In certain cases, aggregation can contribute to the retention of unfolded protein in the ER. Misfolded proteins are often aggregation prone, and their inclusion in large complexes is likely to limit their diffusion through the ER and their insertion into transport vesicles. While in some cases aggre-

gate formation does not hamper the subsequent degradation of the misfolded polypeptide (Molinari et al. 2002), in other cases the ER seems to be unable to efficiently disrupt such complexes, leading to the prolonged accumulation of the misfolded proteins (Sparvoli et al. 2001).

3 An Overview of the ERAD Pathway

In general, the ERAD process can be divided in at least four stages: (i) recognition of the aberrant polypeptide, (ii) dislocation, (iii) release from the ER surface, and iv) degradation. However, it is becoming clear that rather than constituting a single defined pathway, ERAD can be considered as a collection of different pathways that may involve different players depending on the characteristics of the protein substrate in question.

Different mechanisms can contribute to the recognition of misfolded proteins in the ER. The same molecular chaperones that assist the folding of newly synthesized polypeptides have been often implicated in the process that prepares aberrant proteins for degradation. Indeed, chaperone interactions, aside from their value in facilitating protein folding may be important in maintaining proteins in soluble form and/or in targeting any terminally misfolded proteins to the next step of the ERAD pathway (dislocation).

The plant homologue of the immunoglobulin heavy chain binding protein (BiP) is a major ER chaperone of the Hsp70 protein family. Certain ERAD substrates have been found to bind extensively to BiP in the ER, their degradation being tightly associated with their release from the chaperone (Knittler et al. 1995; Skowronek et al. 1998; Chillarón et al. 2000; Molinari et al. 2002). Degradation of some, but not all, ERAD substrates has been found to be impaired in the presence of different BiP mutants (Plemper et al. 1997; Brodsky et al. 1999; Nishikawa et al. 2001), and aggregation of some misfolded proteins has been found to be exacerbated in yeast strains carrying mutated BiP (Nishikawa et al. 2001; Kabani et al. 2003). So, although BiP may play different roles in ERAD, one of them appears to be to maintain particular protein substrates in a soluble, retrotranslocation-competent state.

Different ER oxidoreductases including yeast Eps1p (Wang and Chang 2003), ERp57 (Antoniou et al. 2002), and protein disulfide isomerase (PDI) (Molinari et al. 2002; Tsai et al. 2001) have also been implicated in the disposal of ERAD substrates. Although extensive unfolding may not be strictly required for dislocation (Tirosh et al. 2002; Fiebiger et al. 2002), reduction of disulfide bonds has been shown to precede dislocation of IgM heavy chains (Fagioli et al. 2001) and ER oxidoreductases are obvious candidates as the catalyzers of reducing reactions required to prepare disulphide-bonded substrates for the membrane translocation step (Molinari et al. 2002). However, it should be noted that yeast PDI has been implicated in the disposal of

a cysteine-free polypeptide, indicating that substrate reduction is not the only activity of PDI involved in quality control (Gillece et al. 1999). A physiological redox state is important not only to allow substrate reduction, but also for the general functioning of the ERAD machinery, since a cysteine-free protein is stabilized in the ER by treatments that affect intracellular redox potential or free thiol status (Tortorella et al. 1998). Finally, it is interesting to note that the degradation of certain membrane proteins has been found to require the action of cytosolic chaperones such as Hsp70 and Hsp104p, the latter being a yeast protein belonging to the AAA-ATPase superfamily (Hill and Cooper 2000; Zhang et al. 2001; Taxis et al. 2003). The activity of cytosolic chaperones appears to be specifically required for the disposal of large or tightly folded cytosolic domains of certain membrane proteins (Taxis et al. 2003).

Although interaction with chaperones is clearly important to prepare ERAD substrates for degradation, it is also becoming evident that other factors contribute to divert unfolded proteins from the biosynthetic to the degradative pathway. Within this context, the best characterized recognition mechanism within the ER is the one based on specific modifications of N-linked glycan chains. Many of the proteins that are inserted into the ER are modified by the addition of a core glycan of 14 saccharides (Fig. 1), which is transferred from a lipid carrier to asparagine residues within the sequon Asn-X-Ser/Thr, where X can be any amino acid but proline. The structure of the glycan initially transferred to the protein is the same in virtually all eukaryotes, and this high level of conservation likely reflects a conserved functional role. The large polar structures of core glycans can have a direct effect on protein stability and solubility, but also contribute to protein folding by mediating the interaction of nascent and newly synthesized glycoproteins with two ER chaperones, calnexin and calreticulin (see chapt. by Vitale and Denecke, this volume). While calnexin is a type I membrane protein, calreticulin is a soluble protein. Briefly, these chaperones are related to the legume lectin family and both of them interact with monoglucosylated oligosaccharides produced by the action of ER glucosidase I and glucosidase II (Fig. 1). Most importantly, monoglucosylated oligosaccharides are also produced by the action of UDP-glucose:glycoprotein glucosyltransferase (GT), a folding sensor that can selectively and iteratively add a glucose residue to misfolded (but not to folded) proteins, giving them further opportunities to interact with calnexin and calreticulin, and thus to complete structural maturation.

In several cases, it has been observed that inhibition of glucose trimming, and hence of calnexin/calreticulin binding, accelerates the degradation of misfolded proteins (de Virgilio et al. 1999; Wilson et al. 2000; Chung et al. 2000; Molinari et al. 2002; Mancini et al. 2003). Although this indicates that entry into the calnexin/calreticulin cycle can protect certain substrates from degradation, stabilizing ERAD substrates in their monoglucosylated form not always resulted in a prolonged half-life. While degradation of certain substrates was retarded (Molinari et al. 2002; Cabral et al. 2002; Oda et al. 2003),

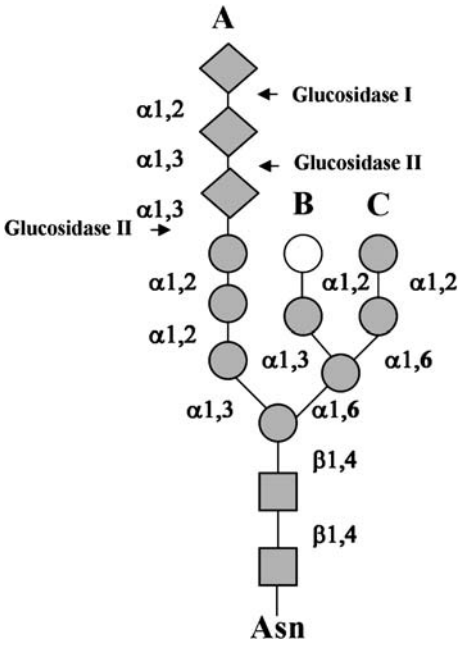

Fig. 1 Structure of the N-linked core oligosaccharide. A branched oligosaccharide composed of 14 units is presynthesized and transferred en bloc to specific asparagine (Asn) residues. The three branches are indicated with the letters A, B and C. The oligosaccharide contains two N-acetylglucosamines (*squares*), nine mannoses (*circles*) and three glucoses (*diamonds*). The site of action of glucosidase I and II are indicated. Yeast and mammalian ER α1,2-mannosidase I preferentially removes the mannose residue present at end of the B branch (*empty circle*)

the degradation of others was left unaffected (Chung et al. 2000; Fagioli and Sitia 2001; Mancini et al. 2003) or even accelerated (Liu et al. 1999). These data would suggest that in many cases glycoprotein degradation can occur both via a pathway involving an interaction with calnexin/calreticulin and via a pathway that does not involve these chaperones. In addition, it appears that some substrates can escape the attention of lectin chaperones and proceed along the ERAD pathway even when glucosidase action is blocked, while others are more sensitive to treatments that favor the accumulation of monoglucosylated glycans. These observations indicate that glucose trimming plays an important but complex role in the ERAD pathway.

The trimming of mannose residues is a further modification of glycan structure that has been implicated in the regulation of ERAD. The ER of yeast and mammals contains a Class I α1,2-mannosidase that removes a mannose from the B-branch of the oligosaccharide to yield the $Man_8GlcNAc_2$ B isomer (Fig. 1). If this process is inhibited using kifunensine or 1-deoxymannojirymicin, or the mannosidase gene is disrupted, degra-

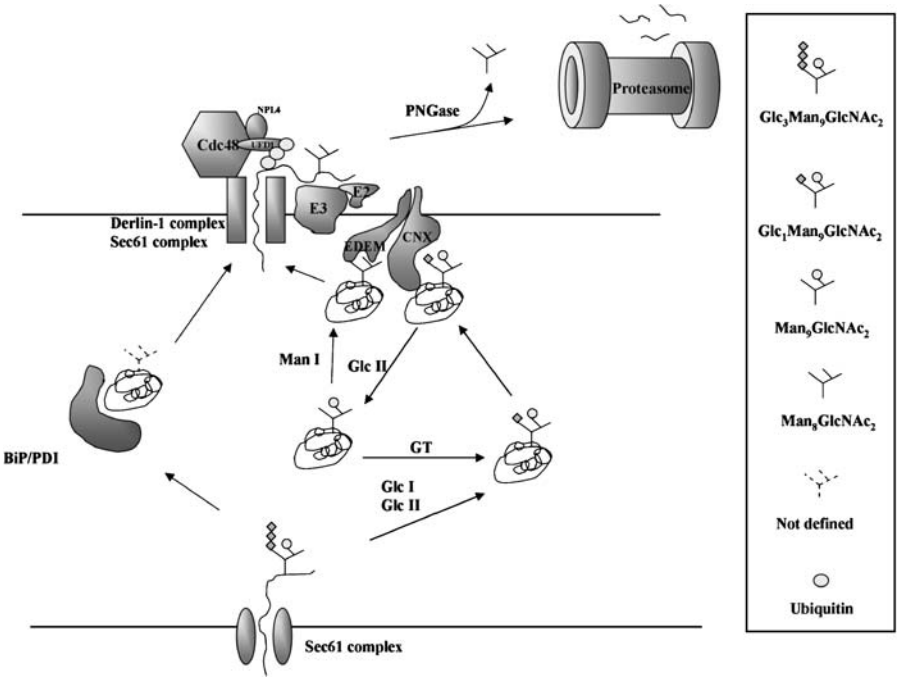

Fig. 2 Schematic model of the ERAD pathway. After being inserted in the ER through the Sec61 channel, proteins fold with the assistance of molecular chaperones and folding enzymes such as BiP and PDI. These folding assistants help maintaining unfolded proteins in solution before they manage to fold or are delivered to the retro-translocation channel. In the case of glycoproteins, folding can be assisted by the lectin chaperones calnexin (CNX) and calreticulin. Although not depicted in the figure, folding substrates can sequentially bind different chaperones. Binding to calnexin and calreticulin requires trimming of glucose residues by glucosidase I (Glc I) and II (Glc II). Further trimming by glucosidase II releases the substrate protein from calnexin. If the released protein fails to fold, it can be reglucosylated by UDP-glucose:glycoprotein glucosyltransferase (GT). Prolonged residence in the ER allows trimming of one or more mannose residues by ER mannosidase I (Man I), and the mannose trimmed glycan mediates binding to EDEM. Since calnexin and EDEM interact, it is possible that substrates are directly transferred from CNX to EDEM. The Sec61 complex and a protein complex containing Derlin-1 have been implicated in the retro-translocation step. Retrotranslocated proteins are ubiquitinylated by the concerted action of ubiquitin-conjugating enzymes (E2) and of ubiquitin-protein ligases (E3). The p97/Cdc48-Ufd1-Npl4 complex is recruited to the ER membrane by the Derlin-1 complex and contributes to the dislocation of the ERAD substrate, which is then escorted to the proteasome for destruction. During these late stages of ERAD, glycoprotein substrates can be deglycosylated by the action of cytosolc peptide:*N*-glycanase (PNGase)

dation of many glycoprotein substrates is impaired (Su et al. 1993; Knop et al. 1996b; Liu et al. 1997; Jakob et al. 1998; de Virgilio et al. 1999; Chung et al. 2000; Wilson et al. 2000; Fagioli and Sitia 2001). Conversely, overex-

pression of ER mannosidase I enhanced the degradation of a glycoprotein substrate (Hosokawa et al. 2003). Even in the case of a substrate that is stabilized by an interaction with calnexin, mannosidase inhibition blocked degradation when glucosidase action (and thus calnexin binding) was also hampered (Wilson et al. 2000). This indicates that the stabilization afforded by inhibition of mannose trimming was not due to enhanced binding to calnexin. So, how do mannosidase inhibitors impede the degradation of glycoprotein substrates? In yeast, deletion of a gene encoding a non essential protein known as homologous to mannosidase I (Htm1p/Mnl1p), selectively causes a reduction in the rate of degradation of mutant glycoproteins, but not of unglycosylated substrates (Jakob et al. 2001; Nakatsukasa et al. 2001). The Htm1p/Mnl1p protein lacks two cysteine residues that are essential for α-mannosidase activity, so it is not involved in the processing of N-linked oligosaccharides. Rather, it is thought to act as a mannose-specific lectin, able to recognize de-mannosylated oligosaccharides. Similarly, an ER degradation enhancing α-mannosidase-like protein (EDEM) that lacks mannosidase activity has been characterized in mammals and its overexpression shown to accelerate degradation of a misfolded protein (Hosokawa et al. 2001). The mammalian protein can complement the yeast orthologue, highlighting the conservation of the quality control system in evolutionarily distant organisms (Gnann et al. 2004). EDEM was found to interact with calnexin, and some substrates appear to be transferred from calnexin to EDEM for degradation (Oda et al. 2003; Molinari et al. 2003). A model has been therefore proposed in which, after release from calnexin, misfolded proteins either reenter the calnexin cycle by the action of GT or are sequestered by EDEM for degradation (Fig. 2). The specificity of the putative lectins remains to be determined but, although $Man_8GlcNAc_2$ was initially thought to represent the main signal for degradation, several lines of evidence indicate that further trimming intermediates may be recognized by the quality control system (Ermonval et al. 2001; Kitzmüller et al. 2003; Frenkel et al. 2003; Herscovics 2001; Herscovics et al. 2002; Hosokawa et al. 2003). It should also be noted that demannosylation per se is unlikely to constitute a signal for degradation since for instance, most glycoproteins are trimmed to $Man_8GlcNAc_2$ in *Saccharomyces cerevisiae*, and mannose-trimmed glycans can be found in long-lived residents of the ER (Matsuoka et al. 1994; Navazio et al. 2002). A cooperation between mannose-binding lectins and ER chaperones may therefore be crucial to confer to the recognition machinery the required specificity toward misfolded glycoproteins. The recent identification of other lectin-like proteins involved in ERAD highlights the complexity of the mechanisms used to target proteins to degradation (Buschhorn et al. 2004; Olivari et al. 2005).

The transient nature of the dislocating polypeptide has so far hampered a detailed characterization of the process by which ERAD substrates cross the ER membrane. Biochemical and genetic evidence indicate that retrotranslocation may occur through the Sec61 complex, the same protein con-

ducting channel that mediates import into the ER. Different ERAD substrates have been found to interact with the Sec61 complex (Wiertz et al. 1996; de Virgilio et al. 1998; Wesche et al. 1999), and the degradation of several substrates is affected in different yeast *sec61* mutants (Plemper et al. 1997; Pilon et al. 1997; Zhou and Schekman 1999; Simpson et al. 1999). However, recent data indicate that the ER membrane contains another protein complex that may play a role in dislocation (Lilley and Ploegh 2004; Ye et al. 2004). This complex contains the membrane proteins Derlin-1 and VIMP (VCP-interacting membrane protein, where VCP is another name for p97/Cdc48, see below), Derlin-1 being the mammalian homologue of the yeast Der1p, a protein that is required for the degradation of a subset of ERAD substrates in this organism (Knop et al. 1996a; Taxis et al. 2003).

In general, dislocation to the cytosol is accompanied by substrate ubiquitinylation, a modification that constitutes a crucial signal for proteasomal degradation. Accordingly, different ubiquitin conjugating enzymes and ubiquitin protein ligases have been shown to be required for the degradation of ERAD substrates (Biederer et al. 1996; Bays et al. 2001; Swanson et al. 2001; Fang et al. 2001; Yoshida et al. 2002; Yoshida et al. 2003b). When polyubiquitination is impaired, many substrates are not dislocated indicating that recognition of the ubiquitin moieties is a pre-requisite for the extraction of misfolded proteins from the membrane (Biederer et al. 1997; Bordallo et al. 1998; de Virgilio et al. 1998; Kikkert et al. 2001; Jarosch et al. 2002). Although ubiquitin chains were initially thought to provide the ratchet for extraction of the protein by proteasomes, accumulating evidence indicates that they may also facilitate recognition by other cytosolic factors that are able to complete the extraction of the dislocating protein. One identified factor is the cytosolic complex containing the AAA-ATPase p97 (also called valosin-containing protein, or Cdc48p in yeast) and its partners Ufd1 and Npl4 (Ye et al. 2001). p97/Cdc48p forms homohexamers that are thought to undergo ATP-dependent movements and can potentially work as an unfoldase, disassembling protein complexes in a fashion that is reminiscent of the disassembly of SNARE complexes by the AAA-ATPase NSF. An interaction with the Derlin-1 complex is likely to place p97 in the right position to take care of ERAD substrates soon after they appear on the cytosolic side of the ER membrane, possibly facilitating their release from interacting proteins (Rabinovich et al. 2002; Ye et al. 2003; Ye et al. 2004) (Fig. 2). p97 can bind substrates independent from ubiquitination, but acts synergistically with the cofactor Ufd1 in the recognition of polyubiquitin chains (Ye et al. 2003). In addition, its yeast homologue Cdc48p has been proposed to cooperate with a set of ubiquitin-binding factors to escort dislocated substrates to the proteasome for degradation (Medicherla et al. 2004; Richly et al. 2005).

As mentioned above, many of the proteins that are inserted into the ER are modified by the addition of N-linked glycans. These glycan chains not only play a role in the recognition within the ER but also contribute

to the identification of the dislocating substrate by ubiquitin ligases able to recognize high-mannose glycan chains (Yoshida et al. 2002, 2003b). Removal of these glycoprotein substrates also involves the action of a cytosolic peptide:*N*-glycanase (PNGase), a deglycosylating enzyme (reviewed in Suzuki and Lennarz 2003). Both the yeast and the mammalian enzymes selectively act on high-mannose glycans and not on complex-type oligosaccharides. In addition, these enzymes act on unfolded glycoproteins but are unable to remove N-linked chains from folded ones (Hirsch et al. 2003, 2004). This enzyme therefore has the requisite activities to act on unfolded polypeptides dislocated from the ER. PNGases from vertebrates and insects also contain a PUB/PUG domain that has been implicated in the association with a variety of ubiquitin/proteasome pathway-related proteins and proposed to recruit a glycoprotein-degradation complex (Suzuki et al. 2001; Suzuki and Lennarz 2003). This domain is not present in the yeast and *Arabidopsis thaliana* homologues (Suzuki et al. 2001), suggesting that different protein complexes may take care of dislocated glycoproteins in different organisms. Although alternative proteolytic systems have been implicated in the degradation of certain substrates (Fayadat et al. 2000; Cabral et al. 2000; Mancini et al. 2003; Brandizzi et al. 2003; Schmitz et al. 2004) the proteasome-mediated destruction of the dislocated protein commonly constitutes the final phase of the ERAD pathway. Since misfolded proteins presented by the dislocation apparatus to the cytosol are likely to rapidly aggregate if left unattended, the dislocation and degradation steps of ERAD are normally tightly coupled, and cytosolic degradation intermediates cannot normally be detected unless proteasomal activity is blocked. This tight link between dislocation and degradation may also explain why certain ERAD substrates accumulate in the ER rather than in the cytosol when proteasomal activity is inhibited (Chillarón and Haas 2000; Mancini et al. 2000).

4 The Emerging Similarities and Differences of the Plant ERAD Pathway

Evidence in favor of the existence of a mechanism of protein retention and disposal in the plant ER was provided by studies on the bean storage protein phaseolin. Phaseolin is a homotrimeric protein of the 7S class that accumulates in the storage vacuoles of bean (*Phaseolus vulgaris*) cotyledons during seed development. Trimer formation is required for the intracellular transport of this protein, and is under a complex regulation involving the interaction with BiP and the trimming of glucose residues from the N-linked glycans (Lupattelli et al. 1997; Foresti et al. 2003). Trimer assembly is mediated by two sets of α-helices present in the monomer, and deletion of either of these domains leads to the synthesis of an assembly-defective protein (Ceriotti et al. 1991; Foresti et al. 2003). When expressed in tobacco protoplasts or transgenic

tobacco plants, assembly-defective phaseolin shows a prolonged association with BiP (Pedrazzini et al. 1994; Foresti et al. 2003), and is degraded by a pathway that is not affected by brefeldin A (BFA), a fungal metabolite that blocks Golgi-mediated traffic to the vacuole (Pedrazzini et al. 1997; Nebenführ et al. 2002). Although it still remains to be established whether cytosolic proteasomes are involved in the eventual degradation of defective phaseolin, it can be concluded that some aspects of the fate of this protein are shared with other ERAD substrates. These aspects include extensive binding to molecular chaperones, retention in the ER and BFA-insensitive degradation.

A second substrate found to be degraded in a BFA-insensitive fashion is the A chain of the plant toxin ricin. Ricin is a ribosome-inactivating protein synthesized by castor bean (*Ricinus communis*) endosperm cells. The mature protein consists of a catalytic chain (RTA) linked by a single disulfide bond and non-covalent interactions to a cell binding B chain (RTB). RTA inactivates ribosomes by specifically depurinating a site in 23S/26S/28S rRNA that is critical for the binding of elongation factors, a modification that leads to an irreversible block in protein synthesis (reviewed in Hartley and Lord 2004). To kill mammalian cells, ricin must therefore cross a cellular membrane to reach the cytosol where its target ribosomes are located. Mammalian cell intoxication begins with the endocytic uptake of ricin holotoxin and its retrograde transport to the ER where the holotoxin is reduced with the help of oxidoreductases present in this compartment (Lord et al. 2003; Spooner et al. 2004). RTA then appears to enter the ERAD pathway and is dislocated to the cytosol (Wesche et al. 1999). Similarly to ricin, other protein toxins have been shown to hijack the ERAD pathway to reach their cytosolic targets (Lord et al. 2003). Clearly, in order to act as cellular poison, a proportion of RTA must uncouple from this pathway to avoid complete destruction by proteasomes, a feature that distinguishes this and other toxins that retro-translocate from the ER from standard ERAD substrates.

In castor bean endosperm cells, ricin is synthesized as a precursor in which the RTA and RTB are connected by a linker peptide of 12 amino acids that has been shown to be required for correct targeting to the vacuole (Frigerio et al. 1998, 2001; Jolliffe et al. 2003). In this context, RTA is not recognized as an ERAD substrate. The ricin precursor is then transported out of the ER and delivered to the vacuole, where a propeptide preceding RTA and the linker peptide are removed, generating mature ricin. When plant cells are forced to express RTA by itself however, the glycosylated RTA initially segregated within the ER lumen becomes cytosolic and is degraded in a BFA-insensitive manner (Frigerio et al. 1998; Di Cola et al. 2001). Treatment with proteasome inhibitors stabilizes RTA expressed in tobacco protoplasts, demonstrating the involvement of this multicatalytic complex in RTA degradation (Di Cola et al. 2001). Although generally following the ERAD pathway, RTA behaves differently to other substrates so far analyzed in yeast and mammalian cells. As mentioned above, ERAD is generally characterized by a tight coupling

between the dislocation and degradation steps, that avoids the transient accumulation of potentially dangerous proteins in the cytosol. In contrast, fractionation experiments could clearly show the accumulation of dislocated RTA in the cytosol even in the absence of proteasome inhibitors, indicating that some specific feature allows this protein to escape prompt recognition by the cytosolic degradative machinery (Di Cola et al. 2001).

Although the ubiquitinylation of the N-terminal amino acid is involved in the degradation of certain substrates in mammalian cells, ubiquitin is normally linked to the substrate through an isopeptide bond between the C-terminus of ubiquitin and a lysine residue of the target protein. Like other toxins that are thought to dislocate from the ER, RTA is characterized by a low lysine content, a characteristic that likely reduces the chances of this protein being ubiquitinylated when appearing on the cytosolic face of the ER. The idea that the transient accumulation of dislocated RTA in the protoplast cytosol was indeed due to the low lysine content of this protein was confirmed by the finding that increasing the number of lysine residues in RTA from two to six was sufficient to convert RTA into a more standard substrate that was unable to accumulate in the cytosol unless proteasomes were inhibited (Di Cola et al. 2005). Consistent with this view was the finding that mutation of the two endogenous lysyl residues present in native RTA caused a marked stabilization of the protein in the cytosol of tobacco protoplasts. Although ubiquitinylation of RTA could not be directly demonstrated, it is likely that this dependence of the rate of degradation on lysine content reflects differential ubiquitinylation of the three RTA forms.

Since an active ubiquitinylation machinery is normally required for the extraction of proteins from the ER, it is unclear how the lysine-free RTA could dislocate as efficiently as the lysine-containing versions of the protein. One possibility is that N-terminal ubiquitinylation is sufficient for RTA extraction, but not for rapid targeting to the proteasome. Alternatively, ubiquitinylation may be required only to release the dislocated protein from the ER surface and not for the actual transfer through the membrane. If this is the case, RTA may have evolved a mechanism to leave the dislocation apparatus without the help of ubiquitin-dependent chaperones. Since ribosomes have been shown to favor RTA refolding in vitro (Argent et al. 2000), it is possible that an interaction between unfolded RTA emerging from the ER membrane and the ribosome triggers toxin refolding and release into the cytosol.

The accumulation of deglycosylated substrates in the cytosol is normally observed only when proteasomal activity is inhibited. This has been taken to mean that deglycosylation and degradation are normally tightly linked, in accord with the observation that PNGase can be present in a complex with the proteasome itself (Park et al. 2001). However, this observation would also be compatible with a model in which glycopeptides are first generated by the proteolytic activities of the proteasomes, and then deglycosylated by a cytosolic PNGase. Since the transfer to PNGase is not strictly required for pro-

tein disposal in yeast and mammals (Suzuki et al. 2000; Blom et al. 2004), it is conceivable that, depending on the level of coupling between dislocation and degradation, PNGase may work either before or after the proteasome along the glycoprotein disposal pathway. The finding that deglycosylated lysine-free RTA accumulated in the cytosol under physiological conditions when proteasomes were active established that deglycosylation can precede proteasomal degradation and suggested that ubiquitinylation plays an important role in the final stages of plant ERAD (Di Cola et al. 2005).

The effects of changing lysine content on RTA degradation also highlighted a difference in the way plant and mammalian cells "see" specific lysine residues and use them to target a protein for degradation. While the lysine-free form of RTA was stabilized and supertoxic in the cytosol of tobacco protoplasts, it retained native potency (when re-associated in vitro with RTB) towards mammalian cells (Deeks et al. 2002). These data suggest that the endogenous lysine residues in RTA do not act as targets for degradation of this protein in mammalian cells, in striking contrast with the observations made from the expression of RTA in plant cells. It is likely that the two lysines have been maintained through evolution precisely because they are not used by mammalian cells as targets for ubiquitinylation. At the same time, we may speculate that their presence in the preproricin sequence, and recognition by the ubiquitinylation machinery, may be advantageous in castor bean endosperm in situations where the preproricin-producing cells may need to deal with truncated forms arising from premature termination of translation or from the translation of aberrant mRNAs (Schubert et al. 2000).

Another protein shown to dislocate to the cytosol in plant cells without being immediately degraded is sGFP-P, a fusion between the green fluorescent protein (GFP) and the P-region of maize calreticulin. In tobacco protoplasts, accumulation of this protein was not enhanced by treatment with BFA or over-expression of Sec12 (which is known to cause a block in the ER-to-Golgi transport), and the protein was slowly degraded via a non-proteasomal pathway (Brandizzi et al. 2003). When this GFP fusion protein was expressed in the epidermal cells of *Nicotiana benthamiana* leaves it was found to label not only the nuclear envelope and the ER (as expected for an ER-targeted protein), but also the cytosol, and was found to be transported into the nucleoplasm in a microtubule-dependent fashion. In the case of this protein, the mechanisms allowing uncoupling of dislocation and degradation remain unclear and analysis of further substrates will be required to determine whether distinct phases of dislocation and degradation are more common in plants that could be expected based on the results obtained in yeast and mammalian cells. Clearly, the finding that the fluorescence emitted by this fusion was evident in the cytosol and nucleoplasm indicated that either dislocation occurred without unfolding of the GFP domain, or that sGFP-P could refold in plant cytosol. While GFP itself can efficiently refold after denaturation, another dislocated substrate containing a GFP moiety was found not to refold

in vitro suggesting that dislocation of folded proteins remains a possibility (Fiebiger et al. 2002).

Studies on the fate of the barley mildew resistance o (MLO) protein have recently helped shed light on the plant ERAD machinery. MLO is an integral membrane protein with seven transmembrane helices, an extracellular N-terminus and a cytosolic C-terminus and normally accumulates in the plasma membrane (Devoto et al. 1999). The MLO protein inhibits a resistance reaction to the powdery mildew pathogen, and a series of *mlo* mutant alleles that confer resistance to this pathogen have been described. Analysis of several mutants revealed that some did not accumulate detectable levels of the MLO protein, whilst having normal levels of transcripts. Analysis of the stability of fusions containing either wild-type or mutated MLO proteins revealed that while the fusions containing the wild-type protein were stable, the ones containing mutants were destabilized, and suggested that the lack of MLO expression was through a quality control mechanism. Accordingly, the degradation of an ustable (MLO-1) allele in Arabidopsis protoplasts was found to be BFA-insensitive and could be slowed down by proteasome inhibitors. Thus, the lack of expression of certain *mlo* alleles is due to a quality control mechanism that impedes expression of the mutant proteins at the cell surface by diverting them for proteasomal degradation (Müller et al. 2005). Alleles that were found to be unstable in Arabidopsis were generally found to be similarly unstable in yeast and human cells, suggesting that the recognition mechanisms are evolutionarily conserved. This work has provided a direct characterization of a component of the plant ERAD machinery, showing that degradation of MLO mutants requires the action of a plant p97/Cdc48 homologue. The Arabidopsis genome contains three homologues of the AAA-ATPase Cdc48/p97 (Lord et al. 2002; Müller et al. 2005). When mutations in the ATPase domains were introduced in one of these genes, and the mutated protein was coexpressed with the unstable MLO-1 fusion protein in Arabidopsis protoplast, degradation of the ERAD substrate was greatly impaired (Müller et al. 2005). This, together with the observation that the MLO-1 protein is ubiquitinylated in vivo, indicates that also in plant cells the Cdc48/p97 protein and ubiquitinylation play an important role in the degradation of certain aberrant proteins.

Besides clearly demonstrating the existence in plants of a pathway analogous to the one described in other systems, the work performed to date has begun to reveal some of the mechanisms that govern substrate recognition, dislocation and degradation in plants. Both phaseolin and sGFP-P are substrates for BiP binding, and this association may be required to maintain these particular proteins in a degradation-competent state. Although only a fraction of assembly-defective phaseolin was ever found in association with BiP, the disappearance of total and BiP-bound defective phaseolin followed similar kinetics, indicating that dissociation from BiP and degradation are kinetically linked (Pedrazzini et al. 1997). A BiP binding site has been mapped

to a region of phaseolin that is also involved in trimer assembly (Foresti et al. 2003). When this region was appended to the C-terminus of GFP it did not disturb protein folding (as judged by the GFP fluorescence) but did stimulate BiP binding. Whether addition of this BiP binding site causes a destabilization of the GFP-phaseolin fusion has not yet been determined.

Whether mannose trimming regulates the degradation of plant glycoproteins is currently not known, and so far the issue has been examined only in the case of RTA. When expressed in tobacco protoplasts, this protein is modified by the removal of one or more mannose residues (Di Cola et al. 2001). Since a mannosidase inhibitor did not affect degradation and since different RTA glycoforms were observed in the cytosol when trimming was undisturbed, it appears that the recognition of RTA as an unfolded protein and its dislocation do not rely on the recognition of a single, specific glycan structure (Di Cola et al. 2005). RTA has been shown to interact with liposomes containing negatively charged phospholipids in vitro, and this interaction is known to induce a conformational change in the protein that may facilitate the recognition of RTA as an ERAD substrate (Day et al. 2002). It is thus possible that RTA may have evolved a specific and glycan-independent mechanism to allow its recognition as an unfolded protein in the ER of intoxicated cells. Clearly, analysis of other glycoprotein substrates will be required to determine the role played by mannose trimming in the plant ERAD pathway.

5
The ERAD Pathway and the Unfolded Protein Response

Besides being equipped with a pathway dedicated to the disposal of misfolded proteins, the ER also contains molecules able to sense the presence of aberrant polypeptides and to trigger a cellular response—the unfolded protein response, UPR. This response invariably includes the increased expression of ER chaperones and folding enzymes and can be triggered by any condition able to compromise folding in the ER. In fact, it can be activated not only by exogenous stresses such as the one imposed by pathogen infection, but also by endogenous ones, such as genetic mutations or the differentiation of professional secretory cells. Experimentally, inhibition of N-glycosylation or treatment with reducing agents are commonly used to induce UPR in different systems, including plants. In developing bean cotyledons, tunicamycin treatment leads to an increase in the synthesis of BiP (D'Amico et al. 1992) and the levels of BiP, PDI, calnexin and calreticulin are increased in different plant tissues exposed to treatments that cause accumulation of misfolded protein in the ER (Denecke et al. 1991, 1995; Koizumi 1996; Koizumi et al. 2001). In addition, genetic mutations that lead to the synthesis of aberrant zein polypeptides cause UPR in developing maize endosperm (Marocco et al. 1991; Coleman et al. 1995; Kim et al. 2004).

While some basic features of UPR have been conserved throughout evolution, plants, yeast and mammals may have developed somewhat different strategies to respond to ER stress. In yeast, ER stress is sensed by the Ire1 protein, a type I transmembrane protein containing a luminal domain that senses ER stress and a cytosolic kinase and endoribonuclease domain. BiP associates to the luminal domain of Ire1p, but when unfolded proteins accumulate the concentration of free BiP decreases and this leads to BiP dissociation, followed by Ire1p oligomerization and autophosporylation. In turn, the cytosolic domain of Ire1p catalyzes the splicing of the *HAC1* mRNA that is then translated into a transcription factor that activates a large set of genes (Okamura et al. 2000; Travers et al. 2000). Genome-wide expression analysis in yeast revealed an intimate link between UPR and ERAD. In addition to the genes encoding molecular chaperones and folding enzymes, many other genes encoding proteins involved in secretion or in the biogenesis of the secretory pathway were found to be up-regulated during UPR (Travers et al. 2000). Among these were several genes known to be involved in ERAD. In addition, the rapid degradation of an ERAD substrate was found to require UPR activation, and UPR was found to be constitutively activated in strains carrying mutations in ERAD components.

In mammals, UPR is under a more complex control. An initial phase during which chaperone genes are induced mainly by the action of the ER-membrane bound transcription factor ATF6, is followed by a second phase during which ERAD gene transcription is activated in a mechanism that involves mammalian homologues of yeast Ire1p and the transcription factor XBP1 (Yoshida et al. 2003a). In addition to Ire1p homologues, the ER of mammalian cells contains an additional transmembrane kinase (PERK), which is similarly activated when BiP dissociates from the luminal sensor domain (Bertolotti et al. 2000). The cytosolic kinase domain of this protein phosphorylates the β-subunit of the translation initiation factor eIF2, causing an inhibition of protein synthesis and the activation of a specific set of genes (Harding et al. 1999; Okada et al. 2002). The first of these effects is thought to reduce the demands made on the ER, and to collaborate with the induction of chaperone synthesis in the recovery from the stress condition.

In plants, BiP transcription is regulated by a feedback mechanism that involves the monitoring of BiP levels (Leborgne-Castel et al. 1999). Homologues of Ire1p are present in Arabidopsis and rice (Koizumi et al. 2001) and a bZIP transcription factor having characteristics similar in part to those of ATF6 has been identified in Arabidopsis (Iwata and Koizumi, 2005). The expression analysis of a large number of Arabidopsis genes led to the identification of UPR induced and repressed genes (Martinez and Chrispeels 2003; Noh et al. 2003). Among the genes induced during UPR were those encoding putative α, β and γ-subunits of the Sec61 translocon, and a putative ubiquitin gene (Martinez and Chrispeels 2003). Since the Sec61 translocon and ubiquitin have implicated in ERAD, it is possible that the induction of these

genes contributes to maintaining an efficient disposal system in the plant ER during stress.

6 Conclusions

While we are still far from a thorough characterization of the ERAD pathway in plants, available information indicates that mechanisms able to extract misfolded polypeptides from the ER lumen and ER membrane are active in plant cells and that the proteasome is in some cases responsible for the degradation of these dislocated polypeptides. The role of ERAD may not be limited to the destruction of structurally defective proteins, and plant cells may exploit this potentially versatile pathway for the fine tuning of certain metabolic processes. It is well established that different cellular pathways including hormonal response, photomorphogenesis and pathogen defense are regulated by the controlled degradation of specific proteins. In addition, the vast array of ubiquitin-protein ligases identified in the Arabidopsis genome suggests the presence of many ubiquitination cascades able to recognize unique degradation signals (Vierstra 2003). By contrast, since ERAD is based on the identification of ill-defined features presented by structurally defective proteins, selective degradation could be achieved by a regulated alteration of the folding state, as in the case of yeast 3-hydroxy 3-methylglutaryl coenzyme A reductase (Hampton 2002; Shearer and Hampton 2005). It is thus possible, if not likely, that ERAD will be found to play a much more pervasive role in plant biology than initially thought.

Acknowledgements We wish to thank Myriam Ermonval for critical reading of the manuscript. Work in the authors' laboratory was supported by MIUR-FIRB grant RBNE01TYZF and by the UK BBSRC and The Wellcome Trust.

References

Ahner A, Brodsky JL (2004) Checkpoints in ER-associated degradation: excuse me, which way to the proteasome? Trends Cell Biol 14:474–478

Antoniou AN, Ford S, Alphey M, Osborne A, Elliott T, Powis SJ (2002) The oxidoreductase ERp57 efficiently reduces partially folded in preference to fully folded MHC class I molecules. EMBO J 21:2655–63

Argent RH, Parrott AM, Day PJ, Roberts LM, Stockley PG, Lord JM, Radford SE (2000) Ribosome-mediated folding of partially unfolded ricin A-chain. J Biol Chem 275:9263–9269

Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY (2001) Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. Nat Cell Biol 3:24–29

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2:326–332

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

Biederer T, Volkwein C, Sommer T (1997) Role of Cue1p in ubiquitination and degradation at the ER surface. Science 278:1806–1809

Blom D, Hirsch C, Stern P, Tortorella D, Ploegh HL (2004) A glycosylated type I membrane protein becomes cytosolic when peptide:*N*-glycanase is compromised. EMBO J 23:650–658

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

Brandizzi F, Hanton S, daSilva LL, Boevink P, Evans D, Oparka K, Denecke J, Hawes C (2003) ER quality control can lead to retrograde transport from the ER lumen to the cytosol and the nucleoplasm in plants. Plant J 34:269–281

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

Buschhorn BA, Kostova Z, Medicherla B, Wolf DH (2004) A genome-wide screen identifies Yos9p as essential for ER-associated degradation of glycoproteins. FEBS Lett 577:422–426

Cabral CM, Choudhury P, Liu Y, Sifers RN (2000) Processing by endoplasmic reticulum mannosidases partitions a secretion-impaired glycoprotein into distinct disposal pathways. J Biol Chem 275:25015–25022

Cabral CM, Liu Y, Moremen KW, Sifers RN (2002) Organizational diversity among distinct glycoprotein endoplasmic reticulum-associated degradation programs. Mol Biol Cell 13:2639–2650

Ceriotti A, Pedrazzini E, Fabbrini MS, Zoppé M, Bollini R, Vitale A (1991) Expression of the wild-type and mutated vacuolar storage protein phaseolin in Xenopus oocytes reveals relationships between assembly and intracellular transport. Eur J Biochem 202:959–968

Chillarón J, Haas IG (2000) Dissociation from BiP and retrotranslocation of unassembled immunoglobulin light chains are tightly coupled to proteasome activity. Mol Biol Cell 11:217–226

Chung DH, Ohashi K, Watanabe M, Miyasaka N, Hirosawa S (2000) Mannose trimming targets mutant α_2-plasmin inhibitor for degradation by the proteasome. J Biol Chem 275:4981–4987

Coleman CE, Clore AM, Ranch JP, Higgins R, Lopes MA, Larkins BA (1997) Expression of a mutant α-zein creates the *floury2* phenotype in transgenic maize. Proc Natl Acad Sci USA 94:7094–7097

D'Amico L, Valsasina B, Daminati MG, Fabbrini MS, Nitti G, Bollini R, Ceriotti A, Vitale A (1992) Bean homologs of the mammalian glucose-regulated proteins: induction by tunicamycin and interaction with newly synthesized seed storage proteins in the endoplasmic reticulum. Plant J 2:443–455

Day PJ, Pinheiro TJ, Roberts LM, Lord JM (2002) Binding of ricin A-chain to negatively charged phospholipid vesicles leads to protein structural changes and destabilizes the lipid bilayer. Biochemistry 41:2836–2843

Deeks ED, Cook JP, Day PJ, Smith DC, Roberts LM, Lord JM (2002) The low lysine content of ricin A chain reduces the risk of proteolytic degradation after translocation from the endoplasmic reticulum to the cytosol. Biochemistry 41:3405–3413

Denecke J, Goldman MH, Demolder J, Seurinck J, Botterman J (1991) The tobacco luminal binding protein is encoded by a multigene family. Plant Cell 3:1025–1235

Denecke J, Carlsson LE, Vidal S, Hoglund AS, Ek B, van Zeijl MJ, Sinjorgo KM, Palva ET (1995) The tobacco homolog of mammalian calreticulin is present in protein complexes in vivo. Plant Cell 7:391–406

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

de Virgilio M, Kitzmüller C, Schwaiger E, Klein M, Kreibich G, Ivessa NE (1999) Degradation of a short-lived glycoprotein from the lumen of the endoplasmic reticulum: the role of N-linked glycans and the unfolded protein response. Mol Biol Cell 10:4059–4073

Devoto A, Piffanelli P, Nilsson I, Wallin E, Panstruga R, von Heijne G, Schulze-Lefert P (1999) Topology, subcellular localization, and sequence diversity of the Mlo family in plants. J Biol Chem 274:34993–35004

Di Cola A, Frigerio L, Lord JM, Ceriotti A, Roberts LM (2001) Ricin A chain without its partner B chain is degraded after retrotranslocation from the endoplasmic reticulum to the cytosol in plant cells. Proc Natl Acad Sci USA 98:14726–14731

Di Cola A, Frigerio L, Lord JM, Roberts LM, Ceriotti A (2005) Endoplasmic reticulum-associated degradation of ricin A chain has unique and plant-specific features. Plant Physiol 137:287–296

Ellgaard L, Helenius A (2003) Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 4:1811–91

Ermonval M, Kitzmuller C, Mir AM, Cacan R, Ivessa NE (2001) N-glycan structure of a short-lived variant of ribophorin I expressed in the MadIA214 glycosylation-defective cell line reveals the role of a mannosidase that is not ER mannosidase I in the process of glycoprotein degradation. Glycobiology 11:565–576

Fagioli C, Sitia R (2001) Glycoprotein quality control in the endoplasmic reticulum. Mannose trimming by endoplasmic reticulum mannosidase I times the proteasomal degradation of unassembled immunoglobulin subunits. J Biol Chem 276:12885–12892

Fagioli C, Mezghrani A, Sitia R (2001) Reduction of interchain disulfide bonds precedes the dislocation of Ig-μ chains from the endoplasmic reticulum to the cytosol for proteasomal degradation. J Biol Chem 276:40962–40967

Fang S, Ferrone M, Yang C, Jensen JP, Tiwari S, Weissman AM (2001) The tumor autocrine motility factor receptor, gp78, is a ubiquitin protein ligase implicated in degradation from the endoplasmic reticulum. Proc Natl Acad Sci USA 98:14422–14427

Fayadat L, Siffroi-Fernandez S, Lanet J, Franc JL (2000) Degradation of human thyroperoxidase in the endoplasmic reticulum involves two different pathways depending on the folding state of the protein. J Biol Chem 275:15948–15954

Fiebiger E, Story C, Ploegh HL, Tortorella D (2002) Visualization of the ER-to-cytosol dislocation reaction of a type I membrane protein. EMBO J 21:1041–1053

Foresti O, Frigerio L, Holkeri H, de Virgilio M, Vavassori S, Vitale A (2003) A phaseolin domain involved directly in trimer assembly is a determinant for binding by the chaperone BiP. Plant Cell 15:2464–2475

Frenkel Z, Gregory W, Kornfeld S, Lederkremer GZ (2003) Endoplasmic reticulum-associated degradation of mammalian glycoproteins involves sugar chain trimming to $Man_{6-5}GlcNAc_2$. J Biol Chem 278:34119–34124

Frigerio L, Vitale A, Lord JM, Ceriotti A, Roberts LM (1998) Free ricin A chain, proricin, and native toxin have different cellular fates when expressed in tobacco protoplasts. J Biol Chem 273:14194–14199

Frigerio L, Jolliffe NA, Di Cola A, Felipe DH, Paris N, Neuhaus JM, Lord JM, Ceriotti A, Roberts LM (2001) The internal propeptide of the ricin precursor carries a sequence-specific determinant for vacuolar sorting. Plant Physiol 126:167–175

Gillece P, Luz JM, Lennarz WJ, de La Cruz FJ, Romisch K (1999) Export of a cysteine-free misfolded secretory protein from the endoplasmic reticulum for degradation requires interaction with protein disulfide isomerase. J Cell Biol 47:1443–1456

Gnann A, Riordan JR, Wolf DH (2004) Cystic fibrosis transmembrane conductance regulator degradation depends on the lectins Htm1p/EDEM and the Cdc48 protein complex in yeast. Mol Biol Cell 15:4125–4135

Hampton RY (2002) ER-associated degradation in protein quality control and cellular regulation. Curr Opin Cell Biol 14:476–482

Harding HP, Zhang Y, Ron D (1999) Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. Nature 397:271–274

Hartley MR, Lord JM (2004) Cytotoxic ribosome-inactivating lectins from plants. Biochim Biophys Acta 1701:1–14

Herscovics A (2001) Structure and function of Class I α1,2-mannosidases involved in glycoprotein synthesis and endoplasmic reticulum quality control. Biochimie 83:757–762

Herscovics A, Romero PA, Tremblay LO (2002) The specificity of the yeast and human class I ER α1,2-mannosidases involved in ER quality control is not as strict as previously reported. Glycobiology 12:14G-15G

Hill K, Cooper AA (2000) Degradation of unassembled Vph1p reveals novel aspects of the yeast ER quality control system. EMBO J 19:550–561

Hirsch C, Blom D, Ploegh HL (2003) A role for N-glycanase in the cytosolic turnover of glycoproteins. EMBO J 22:1036–1046

Hirsch C, Misaghi S, Blom D, Pacold ME, Ploegh HL (2004) Yeast N-glycanase distinguishes between native and non-native glycoproteins. EMBO Rep 5:201–206

Hong E, Davidson AR, Kaiser CA (1996) A pathway for targeting soluble misfolded proteins to the yeast vacuole. J Cell Biol 135:623–633

Hosokawa N, Wada I, Hasegawa K, Yorihuzi T, Tremblay LO, Herscovics A, Nagata K (2001) A novel ER α-mannosidase-like protein accelerates ER-associated degradation. EMBO Rep 2:415–422

Hosokawa N, Tremblay LO, You Z, Herscovics A, Wada I, Nagata K (2003) Enhancement of endoplasmic reticulum (ER) degradation of misfolded Null Hong Kong α_1-antitrypsin by human ER mannosidase I. J Biol Chem 278:26287–26294

Iwata Y, Koizumi N (2005) An Arabidopsis transcription factor, AtbZIP60, regulates the endoplasmic reticulum stress response in a manner unique to plants. Proc Natl Acad Sci USA 102:5280–5285

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

Jakob CA, Bodmer D, Spirig U, Battig P, Marcil A, Dignard D, Bergeron JJ, Thomas DY, Aebi M (2001) Htm1p, a mannosidase-like protein, is involved in glycoprotein degradation in yeast. EMBO Rep 2:423–430

Jarosch E, Taxis C, Volkwein C, Bordallo J, Finley D, Wolf DH, Sommer T (2002) Protein dislocation from the ER requires polyubiquitination and the AAA-ATPase Cdc48. Nat Cell Biol 4:134–139

Jolliffe NA, Ceriotti A, Frigerio L, Roberts LM (2003) The position of the proricin vacuolar targeting signal is functionally important. Plant Mol Biol 51:631–641

Kabani M, Kelley SS, Morrow MW, Montgomery DL, Sivendran R, Rose MD, Gierasch LM, Brodsky JL (2003) Dependence of endoplasmic reticulum-associated degradation on the peptide binding domain and concentration of BiP. Mol Biol Cell 14:3437–3448

Kikkert M, Hassink G, Barel M, Hirsch C, van der Wal FJ, Wierz E (2001) Ubiquitination is essential for human cytomegalovirus US11-mediated dislocation of MHC class I molecules from the endoplasmic reticulum to the cytosol. Biochem J 358:369–377

Kim CS, Hunter BG, Kraft J, Boston RS, Yans S, Jung R, Larkins BA (2004) A defective signal peptide in a 19-kD α-zein protein causes the unfolded protein response and an opaque endosperm phenotype in the maize De*-B30 mutant. Plant Physiol 134:380–387

Kitzmüller C, Caprini A, Moore SE, Frenoy JP, Schwaiger E, Kellermann O, Ivessa NE, Ermonval M (2003) Processing of N-linked glycans during endoplasmic-reticulum-associated degradation of a short-lived variant of ribophorin I. Biochem J 376:687–696

Knittler MR, Dirks S, Haas IG (1995) Molecular chaperones involved in protein degradation in the endoplasmic reticulum: quantitative interaction of the heat shock cognate protein BiP with partially folded immunoglobulin light chains that are degraded in the endoplasmic reticulum. Proc Natl Acad Sci USA 92:1764–1768

Knop M, Finger A, Braun T, Hellmuth K, Wolf DH (1996a) Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast. EMBO J 15:753–763

Knop M, Hauser N, Wolf DH (1996b) N-Glycosylation affects endoplasmic reticulum degradation of a mutated derivative of carboxypeptidase yscY in yeast. Yeast 12:1229–1238

Koizumi N (1996) Isolation and responses to stress of a gene that encodes a luminal binding protein in *Arabidopsis thaliana*. Plant Cell Physiol 37:862–865

Koizumi N, Martinez IM, Kimata Y, Kohno K, Sano H, Chrispeels MJ (2001) Molecular characterization of two Arabidopsis Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases. Plant Physiol 127:949–962

Leborgne-Castel N, Jelitto-Van Dooren EP, Crofts AJ, Denecke J (1999) Overexpression of BiP in tobacco alleviates endoplasmic reticulum stress. Plant Cell 11:459–70

Liu Y, Choudhury P, Cabral CM, Sifers RN (1997) Intracellular disposal of incompletely folded human α1-antitrypsin involves release from calnexin and post-translational trimming of asparagine-linked oligosaccharides. J Biol Chem 272:7946–7951

Liu Y, Choudhury P, Cabral CM, Sifers RN (1999) Oligosaccharide modification in the early secretory pathway directs the selection of a misfolded glycoprotein for degradation by the proteasome. J Biol Chem 274:5861–5867

Lord JM, Ceriotti A, Roberts LM (2002) ER dislocation: Cdc48p/p97 gets into the AAAct. Curr Biol 12:R182–184

Lord JM, Deeks E, Marsden CJ, Moore K, Pateman C, Smith DC, Spooner RA, Watson P, Roberts LM (2003) Retrograde transport of toxins across the endoplasmic reticulum membrane. Biochem Soc Trans 31:1260–1262

Lilley BN, Ploegh HL (2004) A membrane protein required for dislocation of misfolded proteins from the ER. Nature 429:834–840

Lupattelli F, Pedrazzini E, Bollini R, Vitale A, Ceriotti A (1997) The rate of phaseolin assembly is controlled by the glucosylation state of its N-linked oligosaccharide chains. Plant Cell 9:597–609

Mancini R, Fagioli C, Fra AM, Maggioni C, Sitia R (2000) Degradation of unassembled soluble Ig subunits by cytosolic proteasomes: evidence that retrotranslocation and degradation are coupled events. FASEB J 14:769–778

Mancini R, Aebi M, Helenius A (2003) Multiple endoplasmic reticulum-associated pathways degrade mutant yeast carboxypeptidase Y in mammalian cells. J Biol Chem 278:46895–46905

Marocco A, Santucci A, Cerioli S, Motto M, Di Fonzo N, Thompson R, Salamini F (1991) Three high-lysine mutations control the level of ATP-binding HSP70-like proteins in the maize endosperm. Plant Cell 3:507–515

Martinez IM, Chrispeels MJ (2003) Genomic analysis of the unfolded protein response in Arabidopsis shows its connection to important cellular processes. Plant Cell 15:561–576

Matsuoka K, Seta K, Yamakawa Y, Okuyama T, Shinoda T, Isobe T (1994) Covalent structure of bovine brain calreticulin. Biochem J 298:435–442

Medicherla B, Kostova Z, Schaefer A, Wolf DH (2004) A genomic screen identifies Dsk2p and Rad23p as essential components of ER-associated degradation. EMBO Rep 5:692–697

Molinari M, Galli C, Piccaluga V, Pieren M, Paganetti P (2002) Sequential assistance of molecular chaperones and transient formation of covalent complexes during protein degradation from the ER. J Cell Biol 158:247–257

Molinari M, Calanca V, Galli C, Lucca P, Paganetti P (2003) Role of EDEM in the release of misfolded glycoproteins from the calnexin cycle. Science 299:1397–1400

Müller J, Piffanelli P, Devoto A, Miklis M, Elliott C, Ortmann B, Schulze-Lefert P, Panstruga R (2005) Conserved ERAD-like quality control of a plant polytopic membrane protein. Plant Cell 17:149–163

Nakatsukasa K, Nishikawa S, Hosokawa N, Nagata K, Endo T (2001) Mnl1p, an α-mannosidase-like protein in yeast *Saccharomyces cerevisiae*, is required for endoplasmic reticulum-associated degradation of glycoproteins. J Biol Chem 276:8635–8638

Navazio L, Miuzzo M, Royle L, Baldan B, Varotto S, Merry AH, Harvey DJ, Dwek RA, Rudd PM, Mariani P (2002) Monitoring endoplasmic reticulum-to-Golgi traffic of a plant calreticulin by protein glycosylation analysis. Biochemistry 41:14141–14149

Nebenführ A, Ritzenthaler C, Robinson DG (2002) Brefeldin A: deciphering an enigmatic inhibitor of secretion. Plant Physiol 130:1102–1108

Nishikawa SI, Fewell SW, Kato Y, Brodsky JL, Endo T (2001) Molecular chaperones in the yeast endoplasmic reticulum maintain the solubility of proteins for retrotranslocation and degradation. J Cell Biol 153:1061–1070

Noh SJ, Kwon CS, Oh DH, Moon JS, Chung WI (2003) Expression of an evolutionarily distinct novel BiP gene during the unfolded protein response in *Arabidopsis thaliana*. Gene 311:81–91

Oda Y, Hosokawa N, Wada I, Nagata K (2003) EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. Science 299:1394–1397

Okada T, Yoshida H, Akazawa R, Negishi M, Mori K (2002) Distinct roles of activating transcription factor 6 (ATF6) and double-stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) in transcription during the mammalian unfolded protein response. Biochem J 366:585–594

Okamura K, Kimata Y, Higashio H, Tsuru A, Kohno K (2000) Dissociation of Kar2p/BiP from an ER sensory molecule, Ire1p, triggers the unfolded protein response in yeast. Biochem Biophys Res Commun 279:445–450

Olivari S, Galli C, Alanen H, Ruddock L, Molinari M (2005) A novel stress-induced EDEM variant regulating endoplasmic reticulum-associated glycoprotein degradation. J Biol Chem 280:2424–2428

Park H, Suzuki T, Lennarz WJ (2001) Identification of proteins that interact with mammalian peptide:*N*-glycanase and implicate this hydrolase in the proteasome-dependent pathway for protein degradation. Proc Natl Acad Sci USA 98:11163–11168

Pedrazzini E, Giovinazzo G, Bollini R, Ceriotti A, Vitale A (1994) Binding of BiP to an assembly-defective protein in plant cells. Plant J 5:103–110

Pedrazzini E, Giovinazzo G, Bielli A, de Virgilio M, Frigerio L, Pesca M, Faoro F, Bollini R, Ceriotti A, Vitale A (1997) Protein quality control along the route to the plant vacuole. Plant Cell 9:1869–1880

Pilon M, Schekman R, Romisch K (1997) Sec61p mediates export of a misfolded secretory protein from the endoplasmic reticulum to the cytosol for degradation. EMBO J 16:4540–4548

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

Rabinovich E, Kerem A, Frohlich KU, Diamant N, Bar-Nun S (2002) AAA-ATPase p97/Cdc48p, a cytosolic chaperone required for endoplasmic reticulum-associated protein degradation. Mol Cell Biol 22:626–634

Richly H, Rape M, Braun S, Rumpf S, Hoege C, Jentsch S (2005) A series of ubiquitin binding factors connects CDC48/p97 to substrate multiubiquitylation and proteasomal targeting. Cell 120:73–84

Sato M, Sato K, Nakano A (2004) Endoplasmic reticulum quality control of unassembled iron transporter depends on Rer1p-mediated retrieval from the Golgi. Mol Biol Cell 15:1417–1424

Schmitz A, Schneider A, Kummer MP, Herzog V (2004) Endoplasmic reticulum-localized amyloid β-peptide is degraded in the cytosol by two distinct degradation pathways. Traffic 5:89–101

Schubert U, Anton LC, Gibbs J, Norbury CC, Yewdell JW, Bennink JR (2000) Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. Nature 404:770–774

Shearer AG, Hampton RY (2005) Lipid-mediated, reversible misfolding of a sterol-sensing domain protein. EMBO J 24:149–159

Simpson JC, Roberts LM, Romisch K, Davey J, Wolf DH, Lord JM (1999) Ricin A chain utilises the endoplasmic reticulum-associated protein degradation pathway to enter the cytosol of yeast. FEBS Lett 459:80–84

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

Sparvoli F, Faoro F, Daminati MG, Ceriotti A, Bollini R (2000) Misfolding and aggregation of vacuolar glycoproteins in plant cells. Plant J 24:825–836

Spooner RA, Watson PD, Marsden CJ, Smith DC, Moore KA, Cook JP, Lord JM, Roberts LM (2004) Protein disulphide-isomerase reduces ricin to its A and B chains in the endoplasmic reticulum. Biochem J 383:285–293

Su K, Stoller T, Rocco J, Zemsky J, Green R (1993) Pre-Golgi degradation of yeast prepro-α-factor expressed in a mammalian cell. Influence of cell type-specific oligosaccharide processing on intracellular fate. J Biol Chem 268:14301–14309

Suzuki T, Lennarz WJ (2003) Hypothesis: a glycoprotein-degradation complex formed by protein-protein interaction involves cytoplasmic peptide:*N*-glycanase. Biochem Biophys Res Commun 302:1–5

Suzuki T, Park H, Hollingsworth NM, Sternglanz R, Lennarz WJ (2000) PNG1, a yeast gene encoding a highly conserved peptide:*N*-glycanase. J Cell Biol 149:1039–1052

Suzuki T, Park H, Till EA, Lennarz WJ (2001) The PUB domain: a putative protein-protein interaction domain implicated in the ubiquitin-proteasome pathway. Biochem Biophys Res Commun 287:1083–1087

Swanson R, Locher M, Hochstrasser M (2001) A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matα2 repressor degradation. Genes Dev 15:2660–2674

Tamura K, Yamada K, Shimada T, Hara-Nishimura I (2004) Endoplasmic reticulum-resident proteins are constitutively transported to vacuoles for degradation. Plant J 39:393–402

Taxis C, Hitt R, Park SH, Deak PM, Kostova Z, Wolf DH (2003) Use of modular substrates demonstrates mechanistic diversity and reveals differences in chaperone requirement of ERAD. J Biol Chem 278:35903–35913

Tirosh B, Furman MH, Tortorella D, Ploegh HL (2002) Protein unfolding is not a prerequisite for endoplasmic reticulum-to-cytosol dislocation. J Biol Chem 278:6664–6672

Tortorella D, Story CM, Huppa JB, Wiertz EJ, Jones TR, Bacik I, Bennink JR, Yewdell JW, Ploegh HL (1998) Dislocation of type I membrane proteins from the ER to the cytosol is sensitive to changes in redox potential. J Cell Biol 142:365–376

Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P (2000) Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. Cell 101:249–258

Tsai B, Rodighiero C, Lencer WI, Rapoport TA (2001) Protein disulfide isomerase acts as a redox-dependent chaperone to unfold cholera toxin. Cell 104:937–948

Vashist S, Kim W, Belden WJ, Spear ED, Barlowe C, Ng DT (2001) Distinct retrieval and retention mechanisms are required for the quality control of endoplasmic reticulum protein folding. J Cell Biol 155:355–368

Vierstra RD (2003) The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. Trends Plant Sci 8:135–142

Wang Q, Chang A (2003) Substrate recognition in ER-associated degradation mediated by Eps1, a member of the protein disulfide isomerase family. EMBO J 22:3792–3802

Wesche J, Rapak A, Olsnes S (1999) Dependence of ricin toxicity on translocation of the toxin A-chain from the endoplasmic reticulum to the cytosol. J Biol Chem 274:34443–34449

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

Wilson CM, Farmery MR, Bulleid NJ (2000) Pivotal role of calnexin and mannose trimming in regulating the endoplasmic reticulum-associated degradation of major histocompatibility complex class I heavy chain. J Biol Chem 275:21224–21232

Yamamoto K, Fujii R, Toyofuku Y, Saito T, Koseki H, Hsu VW, Aoe T (2001) The KDEL receptor mediates a retrieval mechanism that contributes to quality control at the endoplasmic reticulum. EMBO J 20:3082–3091

Ye Y, Meyer HH, Rapoport TA (2001) The AAA ATPase Cdc48/p97 and its partners transport proteins from the ER into the cytosol. Nature 414:652–656

Ye Y, Meyer HH, Rapoport TA (2003) Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. J Cell Biol 162:71–84

Ye Y, Shibata Y, Yun C, Ron D, Rapoport TA (2004) A membrane protein complex mediates retro-translocation from the ER lumen into the cytosol. Nature 429:841–847

Yoshida Y, Chiba T, Tokunaga F, Kawasaki H, Iwai K, Suzuki T, Ito Y, Matsuoka K, Yoshida M, Tanaka K, Tai T (2002) E3 ubiquitin ligase that recognizes sugar chains. Nature 418:438–442

Yoshida H, Matsui T, Hosokawa N, Kaufman RJ, Nagata K, Mori K (2003a) A time-dependent phase shift in the mammalian unfolded protein response. Dev Cell 4:265–271

Yoshida Y, Tokunaga F, Chiba T, Iwai K, Tanaka K, Tai T (2003b) Fbs2 is a new member of the E3 ubiquitin ligase family that recognizes sugar chains. J Biol Chem 278:43877–43884

Zhang Y, Nijbroek G, Sullivan ML, McCracken AA, Watkins SC, Michaelis S, Brodsky JL (2001) Hsp70 molecular chaperone facilitates endoplasmic reticulum-associated protein degradation of cystic fibrosis transmembrane conductance regulator in yeast. Mol Biol Cell 12:1303–1314

Zhou M, Schekman R (1999) The engagement of Sec61p in the ER dislocation process. Mol Cell 4:925–934