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Search and Destroy: ER Quality Control and ER-Associated Protein Degradation

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ABSTRACT Proteins synthesized in the endoplasmic reticulum (ER) encounter quality control checkpoints that verify their fitness to proceed in the secretory pathway. Molecules undergoing folding and assembly are kept out of the exocytic pathway until maturation is complete. Misfolded side products that inevitably form are removed from the mixture of conformers and returned to the cytosol for degradation. How unfolded proteins are recognized and how irreversibly misfolded proteins are sorted to ER-associated degradation pathways was poorly understood. Recent developments from a combination of genetic and biochemical analyses has revealed new insights into these mechanisms. The emerging view shows distinct pathways working in collaboration to filter the diverse range of unfolded proteins from the transport flow and to divert misfolded molecules for destruction.

KEYWORDS ER quality control, ERAD, protein folding, ubiquitin, proteasome, chaperone, glycosylation, protein degradation

INTRODUCTION

Nearly all proteins secreted from the cell or resident along the secretory pathway begin their journey within the membranes of the endoplasmic reticulum (ER). They cross or integrate into the membrane through a proteinaceous pore called the translocon (Johnson & van Waes, 1999; Meacock *et al.*, 2000; Romisch, 1999). Physically separated from the cytosol, the ER maintains its own distinct lumenal environment for the specific needs of secretory protein biogenesis. An extensive array of factors are on hand to carry out essential maturation steps that can include signal sequence cleavage, N- and O-linked glycosylation, glycosylphosphatidylinositol (GPI)-anchor addition, folding and oligomerization, disulphide bond formation, and isomerization. Since these activities are found nowhere else along the secretory pathway, the uncontrolled flux of immature proteins out of the ER would be disastrous. This scenario is averted by a mechanism termed "ER quality control (ERQC)," which monitors protein folding and assembly and prevents the transport of immature molecules.

The ER must also contend with proteins that become irreversibly misfolded. Errors in transcription and translation, environmental stress (including oxidative damage and unfavorable temperatures), and subunit stoichiometric imbalances are some of the leading causes for erroneous protein biogenesis (Ellgaard & Helenius, 2003; Wickner *et al.*, 1999). Up to 30% of all newly synthesized

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proteins are estimated to be defective (Schubert *et al.*, 2000). Left unchecked, aberrant proteins can be dire to an organism, as they are inherently toxic (Bucciantini *et al.*, 2002). To neutralize them, cells deploy another pathway coupled to ERQC. Termed ER-associated degradation (ERAD), offending molecules are taken out of folding pathways and targeted for degradation.

The flux through the ER includes soluble proteins, single and multi-spanning integral membrane proteins, and lipid anchored proteins. Misfolded proteins that emerge from any of these populations can be eliminated by ERAD. Regardless of species, they are recognized, targeted, and translocated to the ubiquitylation machinery located on the cytosolic face of the ER for modification (most substrates). The molecules are next extracted from the membrane and finally degraded by the 26S proteasome. The process seems simple enough, but the wide variety of substrates made it difficult to imagine that a single pathway could sufficiently monitor all molecules. It is now clear that the task is accomplished through a collaboration of multiple pathways. Investigators are currently undertaking the challenge to delineate these pathways, to understand how the cell arrives at the decision that a protein is misfolded, and how substrates are targeted to the degradative machinery. This review will emphasize these and other emerging concepts and is not intended to be comprehensive. For additional reading, the reader is directed to several excellent reviews recently published (Ellgaard & Helenius, 2003; Kostova & Wolf, 2003; McCracken & Brodsky, 2003; Sitia & Braakman, 2003; Trombetta & Parodi, 2003).

ER QUALITY CONTROL: SORTING AND RETENTION

Proteins synthesized in the ER pose special problems for quality assurance. Soluble molecules can diffuse in the lumenal milieu, whereas more spatially constrained membrane proteins can have domains exposed to the lumen, lipid bilayer, and the cytosol. A quality control system must be able to detect and retain the full range of unfolded proteins while allowing the progress of mature molecules. Since most nascent secretory proteins eventually fold and traffic out of the ER, the mechanisms that comprise ERQC appear to be independent from the decisions to degrade molecules deemed misfolded.

Chaperones: Screeners at the First Line of Defense

Precisely how cells sort and retain unfolded proteins remains unclear. The best-understood mechanism found in higher eukaryotes, the calnexin/calreticulin cycle, integrates activities of both folding and quality control to serve a subset of glycoproteins. Calnexin and calreticulin are homologous lectin chaperones resident in the ER, each with their own substrate preferences. Both bind newly synthesized glycoproteins that contain N-linked glycan(s) trimmed to a single terminal glucose residue by glucosidase I and II. While bound, substrates undergo conformational maturation assisted by a variety of folding enzymes (Frenkel *et al.*, 2004; Molinari & Helenius, 1999; Oliver *et al.*, 1997; Pollock *et al.*, 2004). The process continues until the remaining glucose residue is cleaved by glucosidase II and the substrate is released. Next, a dedicated folding sensor, UDP-glucose:glycoprotein glucosyl transfrease (GT), samples each substrate. Folding intermediates are reglucosylated by GT to allow another round of lectin binding and folding. The substrate specificity of GT is notable, as it recognizes partially structured non-native conformations over native or fully unfolded molecules (Caramelo *et al.*, 2003; Ritter & Helenius, 2000; Trombetta & Helenius, 2000). Through this activity, GT returns folding intermediates to the cycle while misfolded proteins are made available for ERAD (see *An ERAD receptor for carbohydrate signals* below). The proteins that go off cycle can then interact with chaperone proteins that include BiP (an Hsp70 family member) and protein disulfide isomerase (PDI, an oxidoreductase) as a prerequisite for ERAD (Molinari *et al.*, 2004; Molinari *et al.*, 2002).

Nascent proteins that do not use the calnexin/calreticulin cycle encounter other ER chaperones and folding catalysts immediately upon translocation. These factors assist in folding and keep their substrates in the ER in the process (Molinari *et al.*, 2004). In the event that nascent proteins fail to fold or assemble, chaperones play a number of roles to prepare substrates for ERAD. BiP (heavy chain binding protein) is well known for its role in retaining unassembled immunoglobulin heavy chains, for which its name was derived (Haas & Wabl, 1983; Hendershot & Kearney, 1988). This function is widespread; many examples in the literature describe specific interactions between BiP and misfolded ER lumenal proteins. BiP, with its partners Scj1p and Jem1p,

is also needed to prevent substrates from aggregating so that they remain competent for ERAD (Nishikawa *et al.*, 2001). This mirrors the role of its cytosolic homolog, Hsp70, in maintaining the translocation competence of presecretory proteins (Chirico *et al.*, 1988; Deshaies *et al.*, 1988). Indeed, cytosolic chaperones also play key roles in the ERAD of some substrates (see below).

PDI, whose expression is coordinately regulated with BiP by the unfolded protein response (UPR), is also required for the retention and subsequent degradation of a broad range of substrates (Bottomley *et al.*, 2001; Gillece *et al.*, 1999). In mammalian cells, PDI forms mixed disulfides with the β-secretase isoforms that fail to fold following attempts in the calnexin/calreticulin system (Molinari *et al.*, 2002). Another oxidoreductase, ERp44, uses free cysteines in mediating the retention of the Ero1p, a resident ER protein. Erp44 also forms mixed disulfides with unassembled IgM chains to mediate their retention (Anelli *et al.*, 2003). Yet another family member, Eps1p, is required for the retention of misfolded yeast Pma1p (Pma1-D378N) (Wang & Chang, 1999). In strains deleted of the *EPS1* gene, Pma1-D378N traffics efficiently to the plasma membrane but the distribution of normal ER resident proteins is unaltered. Together, these reports show that one major function of lumenal oxidoreductases is to retain misassembled proteins.

Recent evidence suggests that PDI plays the broader role of actively unfolding and targeting substrates to the ERAD pathway. Cholera toxin enters the cell and journeys through the secretory pathway in reverse until it reaches the ER. There, it crosses the membrane to the cytosol, probably through the ER Sec61 pore complex normally used for import. The toxin enters the cell as an A1 subunit (the active domain) covalently attached to the A2 subunit through a disulfide bond. For A1 retro-translocation to occur, the pair must be reduced and unfolded after they enter the ER lumen. Searching for the "unfoldase," Tom Rapoport's group developed and followed a toxin unfolding activity biochemically. The activity co-purified with a single protein, PDI. The unfoldase activity of PDI seems to use an on-off substrate binding cycle typical of other chaperones, except that PDI's is regulated by its redox state rather than ATP binding and hydrolysis (Tsai *et al.*, 2001). Thus, the toxin adapted a pre-existing function of PDI to gain entry to the cytosol where it attacks its target. If misfolded proteins dislocate through the same pore, they would need to unfold fully and to reduce any disulfide bonds that may have formed. By extending PDI's role in cholera toxin dislocation to ERAD, it is a leading candidate in the priming of substrates for export.

A Multifaceted Defense is Deployed Against Aberrant Proteins

The restriction of the calnexin/calreticulin system to a subset of glycoproteins suggested that ERQC is comprised of multiple pathways. This notion, along with the idea that such pathways converge at a common degradation mechanism, emerged from the pioneering studies of two independent groups. From entirely different perspectives, elegant genetic strategies using budding yeast were performed to dissect the regulation and degradation of one of the two yeast HMG-CoA reductase isozymes, Hmg2p, involved in sterol biosynthesis (Hampton *et al.*, 1996), and CPY*, a misfolded variant of the soluble vacuolar peptidase carboxypeptidase Y (Knop *et al.*, 1996). Through these studies, identical components were discovered that are needed for the degradation of both proteins. These included the E3 ubiquitin ligase Hrd1p/Der3p and its associated factor Hrd3p (Bays *et al.*, 2001; Bordallo *et al.*, 1998; Gardner *et al.*, 2000; Plemper *et al.*, 1999), the E2 ubiquitin conjugating enzyme Ubc7p and its associated ER membrane anchor Cue1p (Biederer *et al.*, 1997; Hampton & Bhakta, 1997; Hiller *et al.*, 1996), and the 26S proteasome (Hampton & Bhakta, 1997; Hiller *et al.*, 1996). Together, these studies demonstrated that both membrane and soluble substrates utilize a common core mechanism involving substrate ubiquitylation on the cytosolic face of the ER membrane followed by proteasomal degradation (Figures 1 and 2).

The upstream sorting steps were less clear, but the available genetic evidence suggested the existence of divergent mechanisms. *DER1*, first discovered by Dieter Wolf's group as being required for the degradation of the soluble substrates CPY* and PrA* was found to be entirely dispensable for the membrane substrates Sec61-2p (mutant translocon pore component) and Pdr5p* (mutant membrane transporter ATPase) (Knop *et al.*, 1996; Plemper *et al.*, 1998). Also consistent with these results, *DER1* was absent from the outcome of the *HRD* genetic selection (Hampton *et al.*, 1996). These studies raised the possibility that soluble and membrane proteins define two substrate classes, each with its own

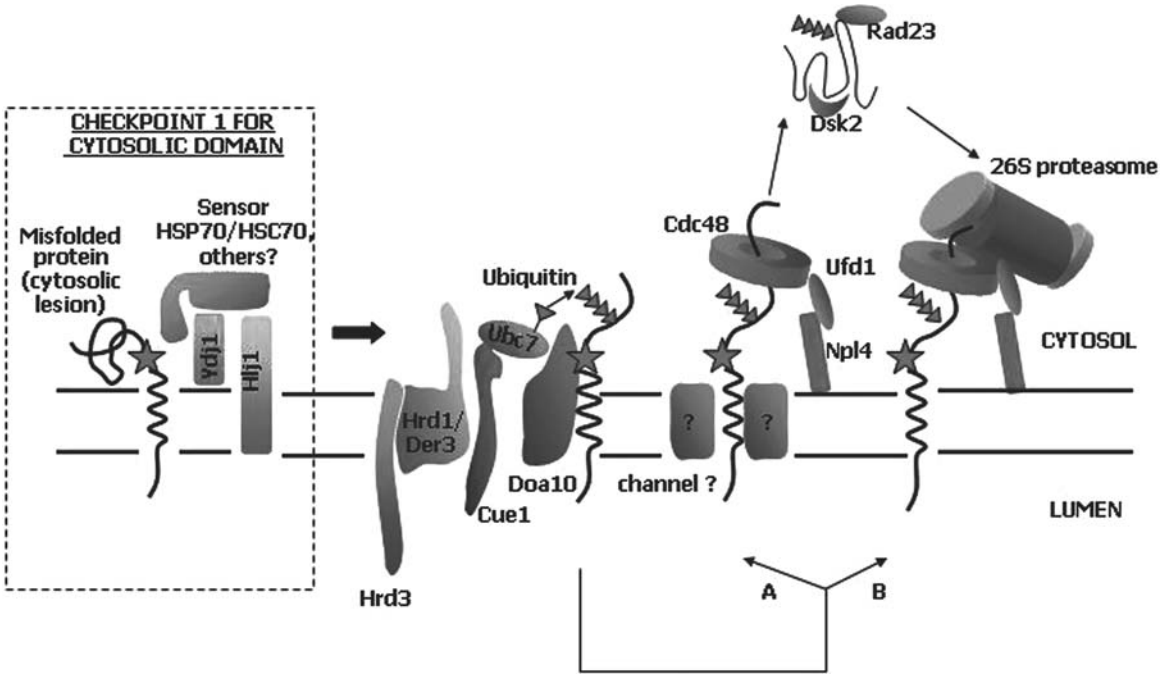

FIGURE 1 ERAD of proteins bearing cytosolic determinants. Misfolded integral membrane proteins with cytosolic lesions are detected by cytosolic chaperone/recognition factors, ubiquitylated, extracted out of the membrane, and degraded by the 26S proteasome as described in the text. The substrate is represented by the black freeform line drawing. The lesion depicted by a star and ubiquitin with triangles. For clarity, not all known factors of each component class is depicted. The translocon is not labeled as it is not clear whether one is required in this pathway.

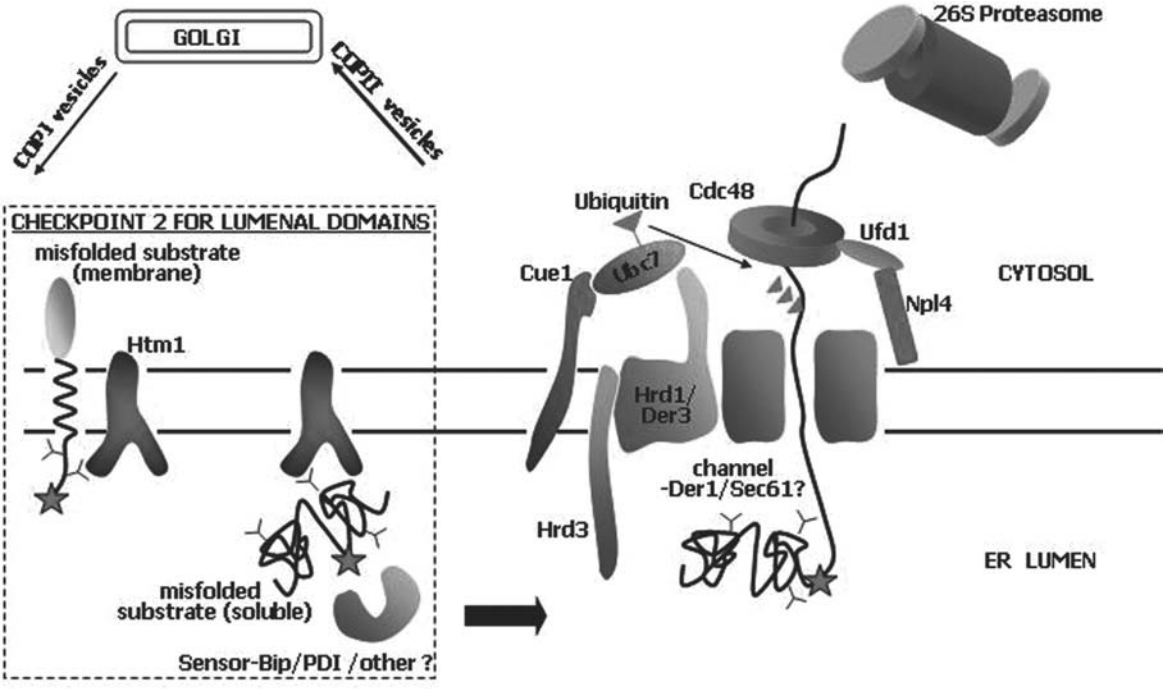

FIGURE 2 ERAD of substrates bearing lumenal determinants. Soluble and integral membrane proteins (depicted by the black freeform lines) with lumenal lesions are detected by lumenal chaperone/recognition factors, targeted to the membrane, dislocated through a protein conducting channel, ubiquitylated, and degraded by the 26S proteasome as described in the text. Stars depict the location of substrate lesions and triangles depict ubiquitin. As in Figure 1, not all factors described in the text are shown for the sake of clarity.

devoted quality control pathways. Further support of the hypothesis came from their distinct requirements for chaperones. The lumenal chaperones BiP, Scj1p, Jem1p, and PDI are required for the degradation of the soluble substrates CPY*, non-glycosylated pro-α factor (pαF), and A1PiZ (mutant variant of the α-1 protease inhibitor). By contrast, the cytosolic chaperones Hsp70 and associated DnaJ-like factors are dispensable (Brodsky *et al.*, 1999; Nishikawa *et al.*, 2001; Plemper *et al.*, 1997). The chaperone requirements are inverted for several membrane substrates, including unassembled Vph1p (subunit of the yeast vacuolar H^+-ATPase), the cystic fibrosis transmembrane conductance regulator (CFTR), Ste6-166p (mutant yeast a-factor transporter), and Sec61-2p (Hill & Cooper, 2000; Huyer *et al.*, 2004; Taxis *et al.*, 2003; Yang *et al.*, 1993; Zhang *et al.*, 2001). The notion of separate pathways for soluble and membrane proteins offered a simple basis for how substrates are "seen" by ER quality control. However, it remained unclear whether the data really reflected distinct pathways or simply different substrate requirements for "accessory" factors within the same pathway.

ER Quality Control: Two Sides of the Same Coin?

Subsequently, combined genetic and biochemical analyses determined at least two quality control systems that work in parallel. In yeast, soluble substrates in the lumen require the maintenance of protein trafficking between the ER and Golgi apparatus, whereas this activity is entirely dispensable for membrane substrates (Caldwell *et al.*, 2001; Taxis *et al.*, 2002; Vashist *et al.*, 2001). The difference suggested retrieval from a post-ER compartment for soluble substrates and static retention for membrane substrates. The notion was confirmed directly by applying *in vitro* vesicle budding assays using purified ER microsomal membranes. CPY* and soluble misfolded viral protein KHN were packaged into COP II (coatamer protein) transport vesicles, while Ste6-166p was completely excluded (Vashist *et al.*, 2001). Whether the trip to the Golgi is a prerequisite for the degradation of soluble substrates is less clear. It has been suggested that the strong stabilization of soluble substrates in protein trafficking mutants could be an indirect effect (Taxis *et al.*, 2002). Thus, substrate recycling might simply reflect the retention mechanism of their associated chaperones PDI and BiP, which are retrieved from the Golgi apparatus via the recycling receptor Erd2p (Semenza *et al.*, 1990).

Although it was clear that ERQC is composed of multiple pathways, hints emerged that substrate fate is not solely determined by soluble versus membrane association. For example, retention in mammalian cells did not follow the trend of yeast. Misfolded or unassembled membrane proteins can utilize either retrieval or static retention mechanisms, although usually not both (Elkabetz *et al.*, 2003; Hammond & Helenius, 1994; Nehls *et al.*, 2000; Yamamoto *et al.*, 2001). Examples of retrieval mechanisms for membrane substrates were also recently reported in yeast. Unassembled Fet3p, a subunit of the plasma membrane iron transporter, is retained through a retrieval mechanism that is dependent on Rer1p (Sato *et al.*, 2004). Previously, Rer1p was known for retaining a subset of ER resident transmembrane proteins including Sec12p (Sato *et al.*, 1995). For its role in ER quality control, Rer1p likely recognizes the unpartnered Fet3p transmembrane segment, much in the way that it retrieves resident proteins (Sato *et al.*, 1996). This example suggests that retrieval in ERQC is not a mechanism devoted exclusively to soluble proteins. However, since unassembled Fet3p is not degraded by ERAD, it is not currently known whether the Rer1p-dependent mechanism is also used by molecules destined for ERAD.

Among ERAD substrates, inspection of lesions pointed to another possible mechanism for sorting. Unlike soluble substrates, statically retained Sec61-2p and Ste6-166p have little exposed in the lumen and have lesions near or in their cytosolic domains (Loayza *et al.*, 1998; Nishikawa *et al.*, 2001). Thus, the site of the lesion could be a major determining factor for sorting. To address the question, a series of ERAD substrates were configured with defined misfolded domains (Vashist & Ng, 2004). An integral membrane substrate called KWW was constructed misfolded only in its lumenal domain (KHN fused to native Wsc1p transmembrane and cytosolic domains). If proteins were sorted simply on the basis of membrane association, KWW would be statically retained. Upon testing, KWW followed the pathway used by the soluble substrates precisely. This and other chimeric molecules demonstrated that the site of lesion could determine the path taken, regardless of membrane association. The pathways were designated ERAD-L (lumenal) and ERAD-C (cytosolic) to emphasize the place where the lesion is detected. The ERAD-C system monitors the cytosolic domains

of membrane proteins (Figure 1) and ERAD-L monitors the lumenal domains of soluble and membrane proteins (Figure 2). Bolstering this view, recent studies from the Michaelis and Brodsky groups showed that the ERAD-L substrate CPY* requires lumenal chaperones while cytosolic chaperones are entirely expendable. Conversely, the ERAD-C substrate Ste6-166p requires cytosolic and not lumenal chaperones (Huyer *et al.*, 2004).

Taken together, these studies show that the ER membrane separates two complementary but distinct quality control systems. They are not equivalent since ERAD-L has the added burden of repatriating substrates to the cytosol. For this function, a glycan-dependent receptor, known variously as EDEM, Htm1p, and Mnl1p, was recently discovered that serves to target substrates for dislocation (see below). In line with the two-system concept, the lumenally oriented receptor is entirely dispensable for ERAD-C substrates, even if they are glycosylated. The pathways that act on the model substrates are ordered sequentially as two checkpoints with ERAD-C preceding ERAD-L. Substrates with strong cytosolic determinants (*e.g.*, Ste6-166p and KWS) use ERAD-C without regard to the state of the lumenal domain (Vashist & Ng, 2004). These studies offered a glimpse of how quality control systems work when handling idealized model substrates.

Unlike idealized substrates, real-world substrates could potentially form boundless conformations such that the location (within a molecule), "strength," and duration of determinants could change over time. Thus, the misfolded side-products of a given molecule could, in principle, utililze different pathways. Studies from the Wolf and Brodsky groups provided some hints to this view. Attachment of CPY* (ERAD-L) to a transmembrane anchor (CT*) bypassed CPY*'s normal requirement for the lumenal factors Der1p and BiP (Taxis *et al.*, 2003). Since CT* lacks a cytoplasmic domain, it seems possible that the context of the fusion created a novel determinant, perhaps in CT*'s particular transmembrane segment, that does not occur when fused to a folded protein. Indeed, lesions in the hydrophilic domains of transmembrane proteins can disrupt the ordering of membrane segments (Chen *et al.*, 2004). Perhaps the best example so far is exemplified by the genetic requirements needed to degrade CFTR in yeast. CFTR is not native to this organism so even the wild type version fails to fold correctly. Compared with other model ERAD substrates, CFTR is degraded very slowly ($t_{1/2} \sim 60$ min) suggesting that it is either structurally difficult to process or does not rapidly form ERAD determinants. Indeed, factors of both lumenal (Htm1/Mnl1p) and cytosolic (Hsp70) quality control systems are needed to degrade CFTR (Gnann *et al.*, 2004; Zhang *et al.*, 2001).

Analyses of membrane protein substrate determinants suggest the existence of an "ERAD-M" (membrane) system. One of the best-characterized determinants for ERAD is the transmembrane segment of the α-subunit of unassembled T-Cell receptors (Bonifacino *et al.*, 1990). However, the fashion by which it is degraded remains unknown. There are indications that HMG-CoA reductase (Hmg2p) levels might be regulated through ERAD monitoring of transmembrane segments. An N-terminal transmembrane domain of Hmg2p was determined to be necessary and sufficient for conferring targeted degradation (Gardner & Hampton, 1999). However, as a functioning protein that is not misfolded, it was not clear how it is recognized by ERAD when sterol levels are low. Hampton and coworkers proposed the provocative hypothesis that Hmg2p undergoes conformational changes that confer an "unfolded" appearance. This idea is quite plausible, since Sec61-2p, which contains a mutation in one of its transmembrane segments, is recognized by ERAD, even though it is functional (Sommer & Jentsch, 1993). The idea was tested using the chemical chaperone glycerol, which has been shown to aid folding and stabilize folded structures. Media supplemented with glycerol stabilized Hmg2p and reduced association with Ubc7p under conditions of regulated degradation (Gardner *et al.*, 2001). Furthermore, experiments showed that chemical chaperones decrease Hmg2p protease sensitivity (Shearer & Hampton, 2004). This finding supports the view that conditional conformational changes could provide the basis of regulated turnover.

Despite the wealth of evidence, it is possible that the lumenal and cytosolic ERQC systems can sufficiently detect proteins with disrupted membrane segments without the need for an "ERAD-M" mechanism. It is possible that structural abnormalities in membrane domains cause subtle but detectable changes in the hydrophilic domains that are sensed by ERAD-L or ERAD-C.

Physiologically, the dynamic nature of ERAD-L prepared a safety valve for the turnover of excess substrate. When ERAD was saturated under conditions of severe stress, CPY* was not retained in the ER but transported to the vacuole (the yeast lysosome) where it was

degraded. This alternative pathway is regulated by the UPR and essential for ER stress tolerance (Spear & Ng, 2003). Increasing the load of misfolded membrane proteins elicits a different response. The overexpression of misfolded Ste6 in yeast leads to their concentration in ER subdomains termed ER-associated compartments (ERACs) (Huyer *et al.*, 2004). The static retention mechanism remains faithful but modified to segregate misfolded proteins until they can be degraded. This mechanism is not unique to Ste6 variants, as CFTR was observed in similar ER subdomains (Fu & Sztul, 2003). In mammalian cells, some substrates localize to a pre-Golgi compartment proposed to be a staging area for dislocation (Kamhi-Nesher *et al.*, 2001). Since the compartments are revealed upon substrate excess, their segregation could be an alternative stress response that prevents harmful interactions until they can be degraded.

For ERAD-C substrates, the precise mechanism of retention is unknown. In the case of Ste6-166p, the protein is excluded from COPII coated vesicles (Vashist *et al.*, 2001). It is now established that cargo proteins leaving the ER are sorted into transport vesicles through interactions with receptors or the vesicle coat protein Sec24p (Belden & Barlowe, 2001; Miller *et al.*, 2003). Given that ERAD-C substrates require the activity of cytosolic chaperones, formation of chaperone complexes in cytosolic domains could easily disrupt the formation of COPII coats. Thus, ERAD-C substrates might be retained by simple exclusion from entering COPII vesicles.

ERAD: Substrate Targeting, Dislocation, and Degradation

One of the key questions to understanding ER stress tolerance is how the decision is made to degrade an ER retained molecule. Simply being unfolded and bound to chaperones are not sufficient criteria. Otherwise, all newly synthesized proteins would be subject to degradation. Once the decision is made, it is manifested by targeting the substrate to the ubiquitylation machinery located on the cytosolic face of the ER. To understand the transition between ERQC and ERAD, protein translocation mechanisms provide excellent paradigms. The principles governing presecretory protein translocation can be useful for framing our views on ERAD (Johnson & Haigh, 2000). In ER import, preproteins are synthesized with signal sequences recognized by receptors (signal recognition particle, Sec61, and Sec62) that direct the nascent polypeptide to the translocon pore (Plath *et al.*, 2004). For soluble proteins, signal sequences are removed by signal peptidase, which precludes their use in ER quality control. Yet, misfolded soluble proteins in the lumenal milieu must be recognized and targeted to the membrane for dislocation to the cytosol. Given that the primary structure of misfolded proteins and their normal counterparts are identical, the signal sequence hypothesis is not directly applicable. How then could a "signal" or determinant be used to differentiate proteins destined for ERAD from other molecules, particularly those in the process of folding?

Counting Carbs to Rid Unwanted Proteins

Recently, a model for substrate targeting involving glycan chains was put forth. Most secretory proteins are modified by branched carbohydrate chains at the sequence Asn-X-Ser/Thr, with X being any amino acid but proline (reviewed in Helenius & Aebi, 2001). Although it was long recognized that N-linked carbohydrates are required for the degradation of glycosylated substrates, their precise function in ERAD was unclear (Knop *et al.*, 1996). Because of their bulky and hydrophilic nature, it was suggested that the modification helps maintain solubility or favorable substrate conformation. The idea that N-linked glycans could be used as ERAD targeting determinants emerged from observations that the trimming of core carbohydrates ($Glc_3Man_9GlcNAc_2$ to $Man_8GlcNAc_2$: Glc, glucose; Man, mannose; GlcNAc, N-acetylglucosamine) was required for efficient degradation (Jakob *et al.*, 1998; Tokunaga *et al.*, 2000). The failure of $Man_9GlcNAc_2$, $Man_7GlcNAc_2$, and $Man_6GlcNAc_2$ glycoforms to substitute for $Man_8GlcNAc_2$implied a degree of specificity expected of a signal:receptor interaction (Jakob *et al.*, 1998). Interestingly, the only other structure with significant activity in yeast, $Man_5GlcNAc_2$, was found on some mammalian substrates *in vivo* prior to degradation (Frenkel *et al.*, 2003). Compared with the rate of trimming by glucosidase I and II, the terminal mannose was removed much more slowly by mannosidase I. This difference led to the proposal of a timing mechanism that would give newly synthesized proteins a fixed period to fold. Should the protein fail to fold after the removal of the first mannose, the $Man_8GlcNac_2$ glycoform would be recognized by a lectin receptor that

targets the substrate to ERAD (Jakob *et al.*, 1998). These studies laid the foundation for carbohydrate-based targeting system for ERAD. The obvious missing link was the lectin receptor.

An ERAD Receptor for Carbohydrate Signals

By mining the *Saccharomyces cerevisiae* genome sequence, two independent groups identified a gene, *HTM1* (homologous to mannosidase I)/*MNL1* (mannosidase-like protein), encoding a protein that fits the criteria for a lectin receptor (Jakob *et al.*, 2001; Nakatsukasa *et al.*, 2001). Htm1/Mnl1p bears 40% sequence similarity to yeast α-mannosidase but does not encode key residues essential for catalysis. Htm1/Mnl1p is an ER resident membrane protein (Nakatsukasa *et al.*, 2001) whose absence does not alter the processing of N-linked glycans (Jakob *et al.*, 2001). Most importantly, it is required for the degradation of the glycosylated substrates CPY*, Pdr5*, and Stt3-7p and dispensable for non-glycosylated substrates Sec61-2p and ΔGpαF.

The importance of the discovery was underscored by the simultaneous discovery of a mammalian ortholog, EDEM (ER degradation-enhancing α-mannosidase-like protein). EDEM was discovered as a UPR-inducible gene by suppression subtractive hybridization suggesting it might play a role in ER stress tolerance (Hosokawa *et al.*, 2001). Surprisingly, the yeast *HTM1*/*MNL1* gene is not UPR-inducible and the significance of the difference between species is unknown (Travers *et al.*, 2000). EDEM overexpression enhances the turnover of misfolded glycoproteins by taking substrates out of the calnexin cycle earlier (Hosokawa *et al.*, 2001; Molinari *et al.*, 2003). EDEM interacts directly with substrate and is associated with calnexin (Molinari *et al.*, 2003; Oda *et al.*, 2003). Reducing EDEM levels resulted in prolonged substrate cycling suggesting that it actively transitions substrates from retention to ERAD. These results show that EDEM works downstream of the calnexin cycle as a post-retention step in targeting substrates to ERAD. Thus, the slow activity of α-mannosidase endows the signal on the substrate for EDEM to remove it from the cycle. However, because known yeast substrates do not use a calnexin cycle for retention (as it lacks GT), Htm1/Mnl1/EDEM must accept substrates from diverse mechanisms. This raises the question of how the putative receptor avoids targeting folded proteins to ERAD, since all glycoproteins are processed to the $Man_8GlcNac_2$ glycoform, at least transiently. How can the system be selective under these circumstances? It is likely that protein determinants exposed only in the unfolded protein work in concert with the $Man_8GlcNac_2$ glycoform. In this way, only those proteins that persist long enough to be trimmed by α-mannosidase and remain unfolded are recognized by the lectin. However, this possibility remains speculative, as such determinants have yet to be uncovered.

Ship 'em out!

Once a lumenal substrate is targeted, it must be moved to the other side of the membrane for degradation. In most transmembrane transport mechanisms, channel-forming protein complexes mediate the process. Because of the topological diversity of ERAD substrates, the identity, or even the general necessity, of a dislocation conduit remains controversial. There is broad agreement, however, that some sort of pore is required for the passage of soluble substrates (Ellgaard & Helenius, 2003; Kostova & Wolf, 2003; McCracken & Brodsky, 2003; Sitia & Braakman, 2003; Trombetta & Parodi, 2003). Whether a pore complex is needed for all transmembrane substrates is less clear. Substrates with disordered cytosolic domains, in principle, can be recognized by chaperones and/or ubiquitylation factors and extracted directly from the membrane for degradation. Alternatively, the passage of transmembrane segments through a translocon pore might be energetically more favorable than "pulling" them directly out of membranes. Membrane substrates with exclusively lumenal lesions would likely utilize a pore complex to initiate the dislocation process since recognition occurs in the lumen (Vashist & Ng, 2004).

The earliest candidate for the dislocation pore was the import pore complex itself. The basic complex is a trimer composed of the Sec61 α, β, and γ subunits (Sec61, Sbh1p, and Sss1p in yeast, respectively) (Görlich & Rapoport, 1993; Panzner *et al.*, 1995). The β, and γ subunits are small single-spanning transmembrane proteins while the α subunit comprises the bulk of the pore with ten transmembrane segments (Van den Berg *et al.*, 2004). Proteins associated with the pore complex including Sec62, Sec63, and the SRP receptor function to mediate targeting activity for imported proteins. No direct interactions between Sec61 and ERAD factors have been reported but a *SEC63* mutant reportedly compromises ERAD suggesting a possible dual role for the factor (Plemper *et al.*, 1997).

The case for a role of the Sec61 complex in protein dislocation was built on findings from several laboratories. The first assessed the environment encountered by an unusual ERAD substrate. The major histocompatibility complex (MHC) class I heavy chains mediate antigen presentation to immune cells. To evade this defense, the human cytomegalovirus encodes the proteins US2 and US11 to target MHC class I heavy-chain proteins for degradation by ERAD (Wiertz *et al.*, 1996). Since the effect is rapid and performed with either protein alone, the US2- or US11-dependent turnover of a single substrate offers a powerful system to dissect the later steps of ERAD. In the presence of US2, Sec61α was found associated with heavy chain (Wiertz *et al.*, 1996). This raised the tantalizing possibility that the import pore does double duty as the "dislocon." Consistent with this idea, a variety of yeast *SEC61* mutants reduced the turnover rate of misfolded protein substrates *in vivo* and *in vitro* (Pilon *et al.*, 1997; Plemper *et al.*, 1997; Zhou & Schekman, 1999). In yeast, deletion of *SSH1*, a non-essential homolog of *SEC61*, results in partial stabilization of CPY* suggesting the possible involvement of a second translocon (Wilkinson *et al.*, 2001). However, since Sec61p is essential for import (and to a lesser extent, its homolog), it is possible that an ERAD factor became limiting due to defective translocation. The observed defects would then be indirect, and the scenario cannot be ruled out in the absence of data demonstrating import competence for all classes of presecretory proteins. At present, Sec61's role in dislocation is not yet firmly established; however, the accumulated data make it a strong candidate.

Recently, a number of studies have raised questions of about Sec61's identity as the dislocation pore. At the very least, the door was opened to other candidates. First, Ploegh and colleagues demonstrated that a tightly folded protein, methotrexate-bound dihydrofolate reductase, when fused to MHC class I HC dislocated efficiently in the presence of US2 or US11 (Tirosh *et al.*, 2003). This finding suggested that the dislocation pore, at least the one used by MHC class I HC, could accommodate a large structure. However, the recent resolution of the archaea SecY translocon structure, which is conserved with the eukaryotic Sec61 complex, makes it difficult to imagine how a fully folded protein could traverse the pore at its narrowest of 5-8 Å (Van den Berg *et al.*, 2004). Earlier work from Art Johnson's lab suggested a different conclusion. Using various molecules to quench fluorescent probes localized within the pore, they derived a maximum limit of 40–60 Å–a size that accommodates many folded proteins (Hamman *et al.*, 1997). Given the extent of conservation (about 50% similarity), could archaea and eukarya translocons really be so different? Even assuming that Sec61 accommodates only extended chains, an export role cannot be ruled out, since ERAD substrates are probably actively unfolded before dislocation (Tsai *et al.*, 2001). Together, these studies raise more questions than answers regarding Sec61. Is there evidence for an alternative translocon for certain substrates? Indeed, two recent studies introduced a new candidate, Derlin-1.

The yeast ortholog of Derlin-1, *DER1*, was the first gene cloned from a genetic screen targeting genes required for the degradation of CPY* and PrA* (Knop *et al.*, 1996). *DER1* is UPR regulated and encodes a protein of 212 residues that consists of four transmembrane segments with the N- and C-termini oriented to the cytosol (Hitt & Wolf, 2004; Travers *et al.*, 2000). In yeast, it is required for ERAD-L substrates and dispensable for ERAD-C substrates (Vashist & Ng, 2004). Thus, it is a specialized factor of ERAD and not one of its core constituents like the proteasome. What role does it play in ERAD?

Devising elegant but entirely different biochemical methodologies, the Rapoport and Ploegh groups demonstrated that ERAD-related events in the ER lumen and cytosol are linked via Derlin-1. Both laboratories exploited the powerful US11/MHC class I heavy-chain system for their analyses. In one approach, the profile of proteins associated with wild-type US11 were compared with a defective US11 mutant (Lilley & Ploegh, 2004). Derlin-1 bound to wild-type US11 and not the mutant suggesting a direct role in US11-mediated dislocation. Indeed, Derlin-1 transiently associates with the substrate and a dominant negative Derlin-1 construct inhibited ERAD. In the other study, a membrane receptor for cytosolic p97 (Cdc48 in yeast) was sought using affinity purification of a digitonin extract of purified microsomal membranes (Ye *et al.*, 2004). p97 is an AAA-ATPase required for the dislocation of all nearly all substrates examined to date (see below). Two polypeptides were identified. The first was identified as Derlin-1, and the second was a novel transmembrane protein termed VIMP (VCP-interacting membrane protein; VCP is another name for p97). VIMP was shown to directly anchor p97 to the ER membrane through its cytosolic domain. VIMP also interacts with Derlin-1

suggesting that VIMP provides the link between Derlin-1 and p97.

Taken together, these studies show that MHC class I heavy chains are targeted to Derlin-1 via US11 through direct interaction. Substrate translocation is assisted by the p97/Ufd1/Npl4 complex through interaction with VIMP and Derlin-1. Missing from the story is the identity of the translocon. Because subunits of the Sec61 translocon were not found associated with any of the components of this system, it led to the proposal that Derlin-1 itself comprises all or part of a translocon pore used by a subset of substrates (Ye *et al.*, 2004). Alternatively, Derlin-1 could primarily function to link targeting factors (US11, Htm1/Mnl1/EDEM) to the Sec61 translocon much the way that the SRP receptor and the Sec62/63 complex adapt the translocon for different modes of import (Gilmore *et al.*, 1982; Plath *et al.*, 2004). Ultimately, identification of the dislocon(s) may require the establishment of a fully reconstituted *in vitro* ERAD system, much like those established for translocation into the ER (Görlich & Rapoport, 1993; Panzner *et al.*, 1995).

AAA ATPases: Providing the Muscle

The forces driving the import of polypeptides into the ER include extrusion of the nascent chain from the ribosome in co-translational translocation and a ratcheting mechanism mediated by BiP/nascent chain complexes in the post-translational mode (Görlich & Rapoport, 1993; Matlack *et al.*, 1999). For the dislocation of ERAD substrates, the AAA ATPase Cdc48/p97 (AAA: ATPases associated with diverse cellular activities) along with its cofactors Npl4 and Ufd1 provide the mechanical energy. AAA ATPases are a large family of enzymes involved a wide array of cellular functions and found in all organisms. Structurally, they form hexameric complexes with a central pore and an amino terminal domain (for review, see Lupas & Martin, 2002). Cdc48/p97 itself is involved in diverse functions that include homotypic membrane fusion, proteolysis, mitotic spindle disassembly, nuclear envelope assembly, and transcriptional activation (DeHoratius & Silver, 1996; Hetzer *et al.*, 2001; Rape *et al.*, 2001). The participation of AAA ATPases in so many cellular functions is due to their underlying activity to unfold and disassemble proteins (reviewed in Lupas & Martin, 2002). As such, it can be easily envision how these enzymes can be adapted to extract or "pull" proteins out of membranes.

Bacterial and mitochondrial AAA ATPases extract membrane proteins prior to proteolysis (Kihara *et al.*, 1999; Leonhard *et al.*, 2000). Thus, it was with great interest that Cdc48/p97 and its cofactors Npl4 and Ufd1 were implicated in the extraction of ERAD substrates to the cytosol. The evidence came simultaneously from different perspectives. The *NPL4* gene was shown to be identical to *HRD4*, a gene required for the regulated degradation of HMG CoA reductase (Bays *et al.*, 2001). The discovery was intriguing since *OLE1* was found as a high copy suppressor of *npl4-1* and *npl4-2* (Hitchcock *et al.*, 2001). The Ole1p enzyme plays a key role in regulating unsaturated fatty acid levels in the yeast ER. The true nature of the connection was brought to light by another study.

OLE1 is regulated by the redundant transcription factors Spt23p and Mga2p (Hoppe *et al.*, 2000). Both factors are initially synthesized as inactive ER transmembrane proteins with the active domains cytosolically oriented. When available fatty acids are low, they are "clipped" from the membrane by a ubiquitin and proteasome dependent reaction and translocated to the nucleus. Npl4p participates in the pathway by forming a complex with its partner Ufd1p as adaptors for Cdc48p (Meyer *et al.*, 2000). Specifically, the Cdc48/Ufd1/Npl4p complex functions to actively liberate the processed and ubiquitylated form of Spt23p (p90) from its membrane bound partner (p120) (Rape *et al.*, 2001). Along similar lines, the complex probably "pulls" HMG CoA reductase from the membrane as a post-ubiquitylation step before it can be degraded by the proteasome. Consistent with this view, the substrate is fully ubiquitylated in a *hrd4-1* allele (Bays *et al.*, 2001).

Direct evidence for Cdc48's role in substrate dislocation came from two studies. Using a cleverly devised semi-intact mammalian cell system (Shamu *et al.*, 2001), Rapoport and colleagues showed that addition of a dominant negative variant of p97/Cdc48 to the system inhibited the dislocation of MHC class I HCs without affecting its ubiquitylation. Correspondingly, yeast mutants of each complex component inhibited the degradation of both the membrane integrated heavy chain and soluble CPY* (Ye *et al.*, 2001). Although the proteasome can unfold proteins before degrading them, Sommer and coworkers showed that it is not required for dislocation. CPY* degradation is impaired in proteasomal mutants but not its ubiquitylation or dislocation. Significantly, this was observed for a mutant of *RPT4*, which encodes a proteasomal AAA ATPase of the 19S

cap (Jarosch *et al.*, 2002). This finding suggests that the Cdc48/Ufd1/Npl4p complex is the only driving force required for the extraction of these substrates from the ER. The findings explain why mutant CFTR accumulated as cytosolic inclusions called aggresomes when proteasomal degradation was inhibited (Kopito & Sitia, 2000).

Whether the substrate is soluble, contains multi- or single transmembrane segments, misfolded lumenally or in the cytosol, or an ERAD-targeted folded protein, the Cdc48/Ufd1/Npl4p complex has been implicated in the degradation of every class (Gnann *et al.*, 2004; Huyer *et al.*, 2004; Jarosch *et al.*, 2002; Rabinovich *et al.*, 2002; Wang & Chang, 2003; Ye *et al.*, 2001). The complex represents a mechanism a step removed from the proteasome through which multiple pathways seem to converge. One notable exception is the aglycosylated precursor protein of the *S. cerevisiae* α factor mating pheromone, called pαF. The substrate is soluble but does not exhibit the same genetic requirements for its degradation as other ERAD-L substrates. Although it is degraded by the proteasome, it does not require ubiquitylation, ER to Golgi transport, Der1p, Htm1p, Hsp70s, Hsp90, or the Cdc48/Ufd1/Npl4p complex (Brodsky *et al.*, 1999; Lee *et al.*, 2004; McCracken & Brodsky, 1996; Pilon *et al.*, 1997; Werner *et al.*, 1996; and our unpublished results). Because of its relatively small size, pαF is thus far the only ERAD substrate amenable to *in vitro* analysis that couples substrate import and export using purified ER microsomal membranes (McCracken & Brodsky, 1996). Using this assay, the Brodsky and Römish groups have shown that the ER chaperones BiP and PDI are both required for pαF dislocation (Brodsky *et al.*, 1999; Gillece *et al.*, 1999). Surprisingly, it also requires the sole yeast calnexin/calreticulin homolog, Cne1p, even though pαF is not glycosylated (McCracken & Brodsky, 1996). Cne1p seems to be dispensable for other yeast soluble substrates like CPY* so pαF degradation represents a distinct ERAD-L pathway (Knop *et al.*, 1996). Indeed, recent data from the Brodsky laboratory have shown that pαF can be exported from the ER using the 19S regulatory cap of the proteasome as the sole driving force (Lee *et al.*, 2004). Subsequent addition of the 20S particle (the proteolytic subunit) resulted in degradation. Taken together, these results show that ERAD substrate dislocation relies on AAA ATPases. Although the Cdc48/Ufd1/Npl4p complex predominates, the 19S regulatory cap of the proteasome can be recruited for some substrates.

Ubiquitin Provides a Handle

The dispensability of the Cdc48/Ufd1/Npl4p complex for pαF was not surprising given it is never ubiquitylated. However, ubiquitylation is a hallmark of all other ERAD substrates examined. We now know that the ubiquitin tag acts not only as a signal for the proteasome but also to promote extraction. Before the implication of p97/Cdc48 in ERAD, it was already established that substrate ubiquitylation is required for dislocation in yeast and mammalian cells (Biederer *et al.*, 1997; Shamu *et al.*, 1999). Moreover, extended ubiquitin chains are required, since incorporation of a ubiquitin K48R mutant, which prevents a specific type of polyubiquitin chain, impairs CPY* dislocation as does expression in a Δ*ubc1*Δ*ubc7* strain, which results in shortened ubiquitin chains (Jarosch *et al.*, 2002). Although Cdc48 can bind unmodified substrate, the Npl4-Ufd1 complex binds polyubiquitin to activate the ATPase activity of Cdc48 to promote the dislocation process (Meyer *et al.*, 2000; Ye *et al.*, 2003). Thus, anchoring the complex to Derlin-1 through VIMP puts it in the "right place at the right time" to capture emerging, newly ubiquitylated substrates. In ERAD, polyubiquitin serves two masters: first, as a signal for substrate extraction from the ER and second, its conventional role in marking substrates for the proteasome.

After Crossing the Border

Once the substrate is cytosolic, it must be delivered to the proteasome. Recent data indicate that an additional step beyond the Cdc48 complex is required for delivery of substrates to the proteasome. Wolf and colleagues devised a clever genetic selection scheme to identify novel ERAD factors. They fused a membrane anchored version of CPY* to the product of the yeast selectable marker *LEU2* and created the reporter protein CTL*. In a *leu2* but otherwise wild type strain, the reporter does not confer leucine prototrophy because it is rapidly degraded. Mutants that stabilize the reporter grow on media lacking leucine and can be easily identified using this phenotype. By screening a yeast genomic deletion collection, strains lacking known ERAD genes scored positively as expected. In addition, two partially redundant factors Dsk2p and Rad23p were identified (Medicherla *et al.*, 2004). This was an exciting discovery since these can bind polyubiquitin chains. In a *dsk2*, *rad23* double mutant, stabilization of CPY* was accompanied by normal ubiquitylation and extraction from

membranes, indicating that these factors function between the Cdc48 and the proteasome. Interestingly, a CPY* variant that never entered the ER was degraded efficiently in the same strain suggesting specificity for ERAD substrates. The authors propose that Dsk2p and Rad23p function together with the Cdc48 complex to deliver substrate to the proteasome.

Once dislocated, glycoproteins are deglycosylated by a cytosolic peptide:*N*-glycanase (Png1p, product of the *PNG1* gene) before they are degraded (Blom *et al.*, 2004; Suzuki *et al.*, 2000). Consistent with a role in processing ERAD substrates, Png1p and the mammalian version of the enzyme exhibit a preference for high mannose glycans (Hirsch *et al.*, 2003). The enzyme also prefers unfolded substrates. Although interesting, the significance is more likely to do with cleavage site accessibility that is common among N-glycosidases. Once out of their normal environment, ERAD substrates would unlikely have folding restored, especially after modification with polyubiquitin.

Substrate Ubiquitylation: The Ticket to the Big House

The basic mechanism of ERAD substrate ubiquitylation is similar to other proteasomal substrates (for reviews, see Goldberg, 2003; VanDemark & Hill, 2002). Ubiquitin (Ub) is activated through formation of an ATP-dependent high-energy thiolester intermediate with an E1 ubiquitin activation enzyme. Next, the ubiquitin moiety is transferred to a cysteine side chain of an E2 ubiquitin conjugating enzyme (Ubc). E2s work together with E3 ubiquitin conjugating enzymes to transfer ubiquitin to the substrate on lysyl residues either directly from the E2 or through an E3-Ub intermediate. For substrates to be degraded, ubiquitin itself becomes ubiquitylated usually at the K48 residue to form polyubiquitin chains.

The earliest clue that non-native ER proteins are degraded by a ubiquitin/proteasomal mechanism came from the observation that the product of an unstable yeast *SEC61* allele, Sec61-2p, could be suppressed by *UBC6* null mutants. *UBC6* encodes an ER membrane anchored E2 with its catalytic domain cytosolically oriented (Sommer & Jentsch, 1993). Shortly after, it was demonstrated that the ΔF506 variant of CFTR is also degraded by a ubquitin/proteasomal mechanism in mammalian cells (Ward *et al.*, 1995). Interestingly, in both cases, the proteins were not grossly misfolded but nevertheless recognized as aberrant. Inhibition of Sec61-2 degradation alone largely restores full translocation activity. This shows that mutant protein is functional and exemplifies the remarkable sensitivity of ER quality control.

Thus far, three yeast E2s have been shown to participate in ERAD. Ubc6p and Ubc7p are ER localized with the soluble Ubc7 protein anchored to the membrane by Cue1p (Biederer *et al.*, 1997; Sommer & Jentsch, 1993). Ubc1p, which has functions in other parts of the cell, seems to augment the other two (Bays *et al.*, 2001; Biederer *et al.*, 1997; Friedlander *et al.*, 2000). The Cue1p-Ubc7p dimer seems to be the most critical, as its loss strongly disrupts the degradation of multiple substrate classes and leads to the constitutive activation of the UPR (Biederer *et al.*, 1996, 1997; Hampton and Bhakta, 1997; Ng *et al.*, 2000). It also collaborates with the yeast E3 proteins Hrd1/Der3p and Doa10p (Bays *et al.*, 2001; Swanson *et al.*, 2001). Both are ER integral membrane proteins containing RING finger domains. Hrd1/Der3p carries the RING-H2 motif and Doa10p contains a recently termed RING-CH motif. In addition to Ubc7p, Hrd1/Der3p has been shown to interact with Ubc1p, and Doa10p with Ubc6p (Bays *et al.*, 2001; Bordallo *et al.*, 1998; Swanson *et al.*, 2001).

The generalized role of E3s in substrate recognition seems to apply for ERAD. Hrd1/Der3p is required for some substrates including Sec61-2p, CPY*, HMG CoA reductase, and KHN, but dispensable for others like Ste6-166p, Pma1p*, and UP* (mutant uracil permease). Doa10p is required for Ubc6p, Pma1p*, Ste6-166p but dispensable for Sec61-2p, CPY*, and KHN (Bordallo *et al.*, 1998; Hampton *et al.*, 1996; Swanson *et al.*, 2001; Vashist *et al.*, 2001; Vashist & Ng, 2004; Wang & Chang, 2003; Wilhovsky *et al.*, 2000). Interestingly, CFTR degradation in yeast requires the combined activity of both E3s (Gnann *et al.*, 2004). Despite these indications, whether the E3 directly recognize substrates or through some intermediary complex is not known. CHIP, a mammalian E3 protein that participates in the ERAD of CFTR is a cofactor of Hsc70 (Meacham *et al.*, 2001). However, CHIP is not devoted to the ERAD pathway as it recognizes a wide variety of cytosolic substrates (reviewed in Wiederkehr *et al.*, 2002). Therefore, the ERAD-C mode may simply be an adaptation of the preexisting protein quality control mechanisms in the cytosol.

Recently, two cytosolic E3 ubiquitin ligases, Fbx2 and Fbs2, were shown to bind N-linked carbohydrates. Expression of dominant negative versions of the

proteins inhibited the turnover of model substrates suggesting a role in ERAD (Yoshida *et al.*, 2002; Yoshida *et al.*, 2003). The discovery was intriguing since N-glycosylated proteins are not normally found in the cytosol and can be easily distinguished from "legitimate" residents. Thus, N-linked glycans would provide the ideal determinants to signal ubiquitylation as substrates emerge from the dislocon. This discovery adds yet another layer to the many strategies deployed by the cell to degraded misfolded secretory proteins.

PERSPECTIVES

From the earliest recognition that unfolded proteins are actively retained in the ER (Gething *et al.*, 1986), the last few years have brought forth an explosion of knowledge from many different fronts. We now know that ER quality control and ERAD are critical homeostatic mechanisms and part of a coordinated stress response that abrogates the toxic effects of misfolded proteins. Not surprisingly, these mechanisms are implicated in the etiology of numerous human diseases including cystic fibrosis, diabetes mellitus, and Parkinson's. In addition, pathogens exploit these pathways to introduce toxins or to evade the immune reponse.

Despite the proliferation of important discoveries over the past few years, it is clear that the overall process is far more complex than previously thought. Lessons learned from a growing cast of substrates show surprising variation in their requirements for ERAD (Brodsky & McCracken, 1999). Indeed, there are even non-native proteins that escape detection by ERQC and caught only in later stages of the secretory pathway (Arvan *et al.*, 2002). Thus, the detection and disposal of aberrant proteins require multiple pathways and numerous factors, both devoted and shared by other cellular processes. As counterintuitive as it might sound, there exists the need to increase the array of substrates being studied. Only then will it be possible to clearly define all the pathways toward the goal of establishing a unifying view of ERQC and ERAD. The call is probably unnecessary, as the field has rapidly grown with investigators joining the effort from many perspectives. In our own laboratory, our studies grew out of our interest in the role of the UPR in cellular stress tolerance (Ng *et al.*, 2000; Spear & Ng, 2003). As authors of this article, it is a bit frustrating to acknowledge, even with the wealth of accumulated knowledge in ERQC and ERAD, that only the surface has been scratched. However, as investigators, we enthusiastically look forward to the exciting developments that are sure to come in the years ahead.

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