ER-associated degradation in protein quality control and cellular regulation

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The ER-associated degradation (ERAD) pathway directs ubiquitin-mediated degradation of a variety of ER-associated misfolded and normal proteins. Recent studies have delineated the molecular machinery responsible for protein ubiquitination and highlighted mechanistic questions surrounding the recognition, extraction and proteasomal destruction of the diverse array of ERAD substrates. Consideration of separate lines of work on this versatile pathway now indicate that despite its central role as an avenue of cellular quality control, ERAD is also harnessed for feedback regulation of sterol synthesis, and most likely numerous other cellular processes. These studies give ERAD a larger role in cellular function, and imply that cellular quality-control pathways could be widely employed in both natural and pharmaceutical control of individual proteins.

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Abbreviations

CFTR	cystic fibrosis transmembrane conductance regulator
CPY	carboxypeptidase Y
DER	degradation in the ER
ERAD	ER-associated degradation
FPP	farnesyl pyrophosphate
HMGR	3-hydroxy 3-methylglutaryl-CoA reductase
HRD	HMG-CoA reductase degradation
SCAP	SREBP cleavage-activating protein
UBC	ubiquitin-conjugating enzyme
UPR	unfolded protein response

Introduction: degradation for quality control or regulation

Recently, there has been an explosion of interest and information about ER-associated protein degradation (ERAD) [1–3]. This degradation process is responsible for the destruction of both integral membrane and lumenal proteins, and functions in protein quality control, where damaged or unfolded proteins are selectively targeted for degradation, while correctly folded ones are spared.

Both normal and misfolded proteins undergo highly specific degradation. Selective degradation of correctly folded proteins underlies cellular regulation of many processes [4]. Examples include degradation of cyclins or their inhibitors to control the cell cycle, destruction of αHIF in response to changing O_2 levels, degradation of IκB to launch innate immunity responses, light-induced proteolysis of phytochrome, or regulated degradation of p53 in both health and disease. In these and innumerable other cases, features that uniquely specify the targeted protein are recognised, usually by ubiquitin ligases, to effect selective degradation.

By contrast, quality-control degradation requires recognition of hallmarks of misfolding shared by a diverse group of proteins with no sequence similarity. Such criteria might seem less useful for cellular regulation of functional cellular proteins. Nevertheless, studies indicate that the essential enzyme 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG-CoA reductase, HMGR) can undergo physiologically regulated degradation by ERAD. Beyond this demonstrated role in sterol regulation, regulation by quality-control degradation is likely to be used in many cellular situations, and may present new avenues for pharmaceutical modulation of protein levels. Specifically, I will discuss new developments in the understanding of ERAD, including work on specific ubiquitin ligases, substrate recognition, retrotranslocation, and the role of this broadly used pathway in the control of sterol synthesis.

HMG-CoA reductase: a regulated ER degradation substrate

HMGR is a rate-limiting enzyme of the mevalonate pathway, by which sterols and a variety of essential isoprenoids are made. In eukaryotic cells, HMGR is controlled, in part, by regulated ER degradation [5–8]. When flux through the sterol pathway is high, degradation rate is high and levels of the protein tend to be low. When flux is low, degradation rate is low and enzyme levels tend to increase. Depending on cell type and signal strength, HMGR half life can vary between >10 h, and <20 min. HMGR is an ER-resident membrane protein, with a highly conserved, catalytic carboxyl terminus attached to a polytopic amino-terminal transmembrane anchor. The transmembrane region is essential for feedback control of stability and will impart regulated degradation to fusion proteins without catalytic activity.

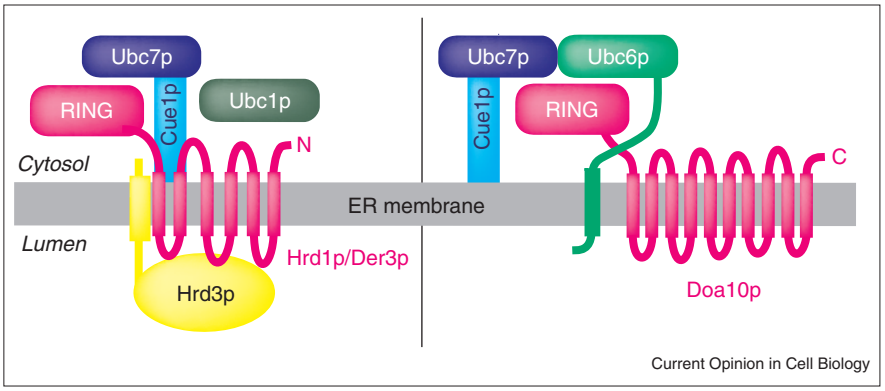
So, what is the mechanism of regulated HMGR destruction, and what is the connection to the ERAD pathway?

Yeast HMG2 degradation: the HRD pathway and ER ubiquitination

The yeast HMGR isozyme Hmg2p undergoes regulated degradation in a manner very similar to the mammalian enzyme. Isolation of *HRD* (HMG-CoA reductase degradation) genes required for HMGR degradation has revealed proteins involved all along the degradation pathway ([9,10••,11•]; N Bays and R Hampton, unpublished data).

Figure 1

Two ER-associated ubiquitin ligases. The Hrd1p/Hrd3p complex is shown on the left, and the Doa10p ligase is shown on the right. The functionally associated ubiquitin conjugating enzymes (E2s) are also shown, along with Cue1p, which anchors Ubc7p to the lumenal face of the ER membrane. In both cases, a multi-spanning RING-domain protein (Hrd1p or Doa10p) is a key component of the complex. In all likelihood, still other ubiquitin E3s are involved in ER degradation of some substrates.



HRD2 encodes the RPN1 subunit of the 26S proteasome, and other *HRD* mutants are alleles of numerous proteasomal genes. Many proteins are targeted to the proteasome by covalent addition of multiple copies of the 7.6 kDa protein tag ubiquitin to form a multiubiquitin chain [4]. Specific ubiquitination is catalyzed by a ubiquitin protein ligase, or E3, that brokers the transfer of ubiquitin from a ubiquitin-conjugating enzyme (UBC, E2) to the target protein or the growing multiubiquitin chain. Hrd1p and Hrd3p are key components of an ER-anchored ubiquitin ligase of the growing RING-motif family of E3s. The cytosolic RING-H2 domain of Hrd1p is required for Hmg2p ubiquitination *in vivo*, and displays ligase activity *in vitro* [10••]. Hrd3p functions in the ER lumen in multiple aspects of Hmg2p degradation, including transmembrane regulation of Hrd1p self-degradation [12•]. Hrd1p and Hrd3p form a complex that mediates interaction of E2 with degradation substrate [13••]. The HRD ligase uses the ER-anchored Ubc7p (Hrd10p) as the principle UBC, and, to a lesser extent, Ubc1p [10••]. Ubc7p is anchored to the ER by Cue1p [2] (Hrd13p). Figure 1 depicts the HRD ligase with relevant E2s. The ubiquitin–proteasome pathway is also responsible for HMGR degradation in mammals [14,15••]. Although the machinery of mammalian HMGR ubiquitination and membrane extraction has not been determined, there are mammalian homologues of most HRD components that are good candidates for the job in larger eukaryotes [16–18].

CPY* degradation and the DER pathway of ER-associated degradation

The genetics of ER quality-control degradation has been analysed by the Wolf laboratory. Their studies focused on CPY*, a mutant version of the normally vacuole-bound carboxypeptidase Y that is retained in the lumen of the ER, where it is degraded with a half life of around 20 minutes. Genes for CPY* degradation in the ER (DER) have been identified, revealing the unanticipated role of the cytosolic ubiquitin–proteasome pathway for the degradation of this demonstrably lumenal substrate [19,20]. Despite the conceptual disparity between quality control destruction of CPY* and regulated degradation of Hmg2p, the same machinery is employed for ubiquitination. The encoded DER proteins include Der3p, which is identical to Hrd1p [21••], Hrd3p [22], the E2s Ubc7p (Der2p [19]) and Ubc1p [23••], as well as several proteins specifically required only for lumenal substrates (e.g. Der1p). Work by numerous laboratories has shown that this pathway degrades a variety of misfolded or unassembled proteins with no similarity in sequence [24,25,26•].

These studies using model substrates imply that the HRD/DER pathway is used for degradation of naturally arising misfolded proteins. This idea is supported by the observations that *hrd1*- or *hrd3*-null mutants are more sensitive to applied ER stresses, and have higher levels of unfolded ER proteins in the absence of stress, as measured using reporters regulated by the unfolded protein response (UPR), a signaling pathway that is keyed to levels of lumenal misfolded proteins [23••,27••]. This latter result is particularly intriguing because it implies that unfolded protein levels in the ER are kept in check by continuous degradation of misfolded proteins — either nascent ones that partition into the degradation pathway while still unfolded, or those that become misfolded for other reasons.

More recent work has led to the realisation that ERAD is complex and multifaceted. Below, I discuss and delineate some of the new findings of this burgeoning field, including the unexpected use of ERAD for the regulation of HMGR and probably other proteins.

Between ubiquitination and the proteasome: Cdc48 and retrotranslocation

Removal of both membrane proteins and purely lumenal ERAD substrates presents a significant energy barrier. A variety of current studies focus on a molecular complex that appears to manage this problem. The more recently characterized Hrd4p functions after Hmg2p ubiquitination, promoting delivery of the ubiquitinated Hmg2p to the proteasome [11•]. Hrd4p is identical to Npl4p, and exists in a complex with AAA-ATPase Cdc48p and Ufd1p

to promote proteasomal degradation or processing of a growing number of ubiquitinated substrates in mammals and yeast [11•,28•–33•]. The Cdc48p–Npl4p–Ufd1p complex has been postulated to effect removal of ubiquitinated proteins from the ER and/or multiprotein complexes (see [34] for a more detailed review).

The ER translocation channel Sec61p has also been proposed to be involved in retrotranslocation of ERAD substrates from the ER compartment, providing a route for peptide exit [35–37]. This might be fuelled by Cdc48p ATPase activity. Although evidence for the role of Sec61p is not definitive, this reasonable possibility will certainly be an ongoing focus that will lead to more mechanistic resolution.

Other ER ubiquitin ligases: a growing group of guardians

The HRD/DER complex and its ancillary proteins can degrade an impressive variety of substrates. However, there are numerous misfolded proteins that are ubiquitinated by the ER-associated E2 Ubc7p in a Hrd1p-independent manner. These non-Hrd1p substrates include mutant ER-retained uracil permease [25], unassembled Vph1p [38], and mammalian CFTR (cystic fibrosis transmembrane conductance regulator) expressed in yeast [39].

These exceptions indicate that other ubiquitin ligases participate in ERAD. In fact recently, a new yeast ER-resident multi-spanning ubiquitin ligase, Doa10p, has been cloned and characterized [40••]. Like Hrd1p, Doa10p has a role in ERAD, as indicated by increased UPR signaling in *doa10*-nulls. In contrast to Hrd1p, Doa10 uses E2s Ubc7p and Ubc6p, and its model substrates are Hrd1p-independent (Figure 1). Doa10p and Hrd1p appear to work together to maintain acceptable levels of misfolded ER proteins: loss of both proteins causes a larger UPR than loss of either alone. Interestingly, the ER degradation of Ole1p has been reported to be unaffected by loss of either Hrd1p or Doa10p [28•], implying that other ER E3s await discovery.

How are misfolded proteins recognised for quality control degradation?

The mechanism of misfolded protein recognition in ERAD remains an open question. It is not surprising that chaperones have been implicated in several studies. It appears that the lumenal heat shock protein 70 (Hsp70) Kar2p may be more important in degradation of lumenal ERAD substrates [35], whereas the four cytosolic Hsp70s, Ssa1–4, play a more significant role in degradation of integral membrane substrates [39]. Only a small number of substrates have been examined so far, however.

In recent studies, a chaperone-containing ubiquitin ligase was described. It is formed by mutual binding of the U-box protein CHIP with a chaperone and an E2, leading to a complex that specifically ubiquitinates chaperone-recognised misfolded proteins [41•–43•], including the ER membrane protein CFTR [44••]. A similar role for chaperones (i.e. substrate recognition) may exist in ERAD ligases as well. It is interesting that the Hrd3p lumenal domain has numerous tetratricopeptide repeats, independently implicated in interactions between chaperones and various binding partners [45]. Conversely, it may be that the ligase subunits themselves participate in recognition of misfolded proteins. The Hrd1p cytosolic RING domain has the intrinsic capacity to ubiquitinate a model misfolded protein *in vitro* [10••], a behaviour that might reflect an aspect of chaperone-independent identification of quality-control substrates.

Is Golgi function required in ER-associated degradation of some proteins?

Several laboratories have made the surprising discovery that the route of ER degradation taken by soluble lumenal proteins and ER-membrane-anchored proteins may be different. Experiments using mutants deficient in secretory pathway traffic [26•,46•], and direct biochemical studies using *in vitro* budding assays [26•], indicate that lumenal proteins may require a visit to the Golgi compartment before their destruction by ERAD. These authors propose that lumenal proteins must be cycled through the Golgi compartment, and then retrieved to the ER before being competent for degradation. By contrast, integral membrane ERAD substrates do not generally have this requirement, although not enough substrates have been tested to strengthen this generalization. Genes required for aspects of this process — *BST1* [26•], *ERV29* and *ERV14* [46•] — have been implicated previously in aspects of ER–Golgi transport, although *BST1* appears to be involved specifically in transport of misfolded proteins, adding another process where recognition of features of misfolding occurs.

At present, it is not clear what features of a Golgi visit would render substrates ERAD-competent. One possibility is that some sort of glycosylation or processing of carbohydrates must occur. Alternatively, it could be that other Golgi modifications are involved, or that the retrotranslocation process itself allows efficient presentation of lumenal substrates to the ERAD machinery. However, another interpretation cannot be ignored [47•]. Mutants deficient in ER-to-Golgi cycling will most certainly have effects on the ER compartment, and far more work must be done to decide if these observations are reflecting a deficiency in ER function or a *bone fide* requirement for Golgi visitation for lumenal ERAD. Whatever the mechanism, these new observations make ER degradation a far more dynamic process, allowing for a whole new layer of stability regulation based on altering ER exit or return.

Regulation of Hmg2p: HRDing a normal protein into the not-OK corral

The ongoing body of work on many aspects of ERAD shows that unfolded proteins can undergo constitutive degradation by a variety of mechanisms. However, unlike any of the typical ERAD substrates, HRD-dependent degradation of Hmg2p is subject to physiological feedback

control. This exception is interesting in its own right, by virtue of the connection to cholesterol, but also indicates the strong possibility that ER quality-control pathways are involved in unknown aspects of cellular regulation.

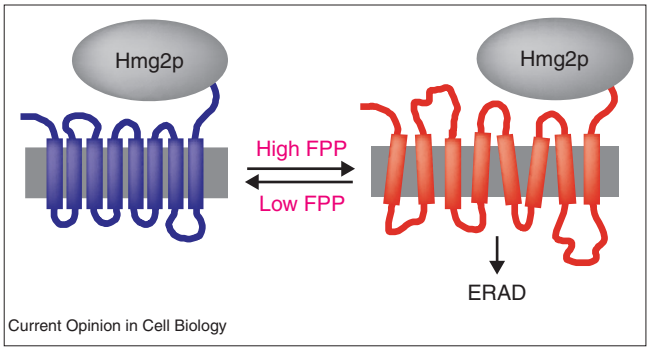
Hmg2p ubiquitination is positively regulated by the 15-carbon mevalonate pathway intermediate farnesyl pyrophosphate (FPP), or a molecule derived from it [48]. Altering cellular FPP levels causes parallel alterations in Hmg2p degradation rate, and this regulation requires Cod1p/Spf1p, an ER-localized P-type ATPase [49,50]. Maximal response to FPP requires an oxysterol in yeast as well [51]. Similarly, mammalian HMGR degradation is keyed to levels of an FPP-derived signal that works in concert with sterols [52,53]. Regulated degradation of Hmg2p is a unique property of the protein, and is highly sensitive to alterations in sequence [54]. Other HRD substrates, including damaged versions of Hmg2p, undergo constitutive, unregulated degradation. The entire pool of Hmg2p is subject to regulated entry into the HRD pathway, and mature protein can be rapidly mobilized to enter the HRD pathway by elevating FPP [8].

The structural transition model for regulated ERAD

How is Hmg2p allowed regulated entry into a quality-control pathway? The most direct model is that Hmg2p can adopt or reveal the structural features of a quality-control substrate when degradation signals are high (Figure 2). This idea was tested by adapting an approach used in the study of CFTR ΔF508, the major disease-causing form of the cystic fibrosis protein CFTR. CFTR ΔF508 is functional, but folds so slowly that it is degraded by ubiquitin-mediated ER degradation before it can attain a degree of folding maturity sufficient to exit the ER and move to the plasma membrane. The folding defect of CFTR ΔF508, and the resulting lack of cell surface chloride ion conductance, can be alleviated in living cells by treatment with the chemical chaperone glycerol [55,56]. This class of structurally diverse compounds promotes the folding of proteins *in vitro* and *in vivo* [57].

If Hmg2p is recognised as a quality-control substrate, then glycerol might be expected to similarly stabilize this protein in living yeast cells. In conditions where signals for degradation are high, glycerol and other chemical chaperones cause rapid (5 min) stabilization of Hmg2p or the regulated Hmg2p–GFP reporter [13••]. The extent of stabilization caused by glycerol is identical to that observed when physiological signals for degradation are lowered with drugs, and the maximal effects of glycerol and physiological stabilization are not additive. Glycerol treatment affects neither the HRD machinery nor the mevalonate pathway. These simple studies engender several more refined questions. For example, are there directly observable changes in Hmg2p structure that can be observed by manipulating the signaling pathway, or by applying chemical chaperones? Is the stabilized state caused by glycerol similar to that caused by lowering FPP-derived signals? How does FPP or its derivatives cause this change? Limited proteolysis studies on microsomal Hmg2p appear to be a promising avenue [13••], and are being refined to best address this 'structural transition hypothesis'.

Figure 2



The structural transition model for regulated degradation of Hmg2p. In cells with high levels of FPP, Hmg2p is recognised as an ERAD quality-control substrate by the HRD/DER machinery. Lowering FPP levels or glycerol cause a rapid, reversible stabilization of Hmg2p. Although the structural change is depicted as graphically dramatic, it may be that subtle determinants are presented or unveiled when FPP is high, triggering recognition as an ERAD substrate. Regulated transitions to ERAD or other quality-control substrates have broad potential as both undiscovered mechanisms in normal cellular regulation and as undeveloped axes for chemical modulation of individual proteins *in vivo*.

This model of yeast Hmg2p regulation has parallels to mammalian HMGR. It has been suggested that mammalian HMGR is susceptible to ERAD when monomeric, is stable when a dimer, and that this transition is physiologically regulated [58]. This can be viewed as regulated transition to a quality control structure, because many quality control substrates are unassembled (monomeric) subunits of multimeric structures. In fact, it could be that the same occurrence underlies the HRD-sensitive state of Hmg2p.

Biological and medical implications of regulation by quality control

The programmed entry of Hmg2p into the ER quality-control pathway is an example of a mode of protein regulation that could occur in many circumstances. Considering the number of separate routes by which ERAD appears to occur, it is quite possible that other proteins are similarly regulated. It has been reported that yeast Δ9 fatty acid desaturase, or Ole1p, undergoes regulated degradation in the ER membrane, and is thus a reasonable candidate for regulated ERAD [28•].

The idea of regulation by ER quality-control mechanisms can transcend degradation. Mammalian SREBP cleavage-activating protein (SCAP), which shares with both HMGR and, to a lesser extent, Hmg2p a transmembrane motif known as the sterol-sensing domain, is retained in the

mammalian ER when sterol levels are elevated [59]. This sterol-triggered retention is central to SCAP-mediated regulation of the SREBP transcriptional regulator. Although degradation is not the final response, ER retention is another frequently described cellular response to misfolded proteins. Perhaps SCAP undergoes a sterol-mediated structural transition that renders it susceptible to retention by ER quality-control mechanisms?

Cellular use of quality-control pathways need not be restricted to the ER. Selective destruction of misfolded proteins occurs in numerous eukaryotic cellular compartments, and in prokaryotes, and each specific pathway has the potential for regulatory employment.

The example set by Hmg2p also suggests a general strategy for controlling proteins by pharmaceutical means. In the broadest sense, a molecular signal causes HMGR to become susceptible to quality-control degradation. Perhaps it is possible to discover small molecules that, upon specifically binding to a target protein, similarly cause that protein to present or acquire features of a quality-control substrate and undergo selective, rapid degradation. Considering the ubiquity of quality-control pathways, this highly specific strategy of protein targeting could be very generally effective if systematic ways to discover and develop such 'protein quality antagonists' can be devised. However, working out the detailed mechanisms involved in recognition and destruction of quality-control protein is a *sine qua non* for the eventual realisation of this novel strategy.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Plemper RK, Wolf DH: **Endoplasmic reticulum degradation. Reverse protein transport and its end in the proteasome.** *Mol Biol Rep* 1999, **26**:125-130.

2. Sommer T, Wolf DH: **Endoplasmic reticulum degradation: reverse protein flow of no return.** *FASEB J* 1997, **11**:1227-1233.

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

4. Ciechanover A, Schwartz AL: **Ubiquitin-mediated degradation of cellular proteins in health and disease.** *Hepatology* 2002, **35**:3-6.

5. Kumagai H, Chun KT, Simoni RD: **Molecular dissection of the role of the membrane domain in the regulated degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase.** *J Biol Chem* 1995, **270**:19107-19113.

6. Chun KT, Simoni RD: **The role of the membrane domain in the regulated degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase.** *J Biol Chem* 1992, **267**:4236-4246.

7. Hampton RY, Rine J: **Regulated degradation of HMG-CoA reductase, an integral membrane protein of the endoplasmic reticulum, in yeast.** *J Cell Biol* 1994, **125**:299-312.

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

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

10. •• Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY: **Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation.** *Nat Cell Biol* 2001, **3**:24-29.
This and Gardner *et al.* (2000) [12•] demonstrate that Hrd1p is the rate-limiting ubiquitin ligase for degradation of Hmg2p (and other ER proteins) in yeast, and that Hrd1p, with Hrd3p, forms a transmembrane ubiquitin ligase that functions on both sides of the membrane. See also Kumagai *et al.* (1995) [5] and Gardner *et al.* (2001) [13••].

11. • Bays NW, Wilhovsky SK, Goradia A, Hodgkiss-Harlow K, Hampton RY: **HRD4/NPL4 is required for the proteasomal processing of ubiquitinated ER proteins.** *Mol Biol Cell* 2001, **12**:4114-4128.
This, together with [28•–33•], all released in a few months of each other, place a new component, the Cdc48 AAA ATPase and its complex partners, in the 'middle' of the ER degradation pathway, between ubiquitination and proteasomal degradation or processes [31•,33•], both in yeast and in mammals [30•].

12. • Gardner RG, Swarbrick GM, Bays NW, Cronin SR, Wilhovsky S, Seelig L, Kim C, Hampton RY: **Endoplasmic reticulum degradation requires lumen to cytosol signaling. Transmembrane control of Hrd1p by Hrd3p.** *J Cell Biol* 2000, **151**:69-82.
See annotation Bays *et al.* (2001) [10••].

13. •• Gardner RG, Shearer AG, Hampton RY: ***In vivo* action of the HRD ubiquitin ligase complex: mechanisms of endoplasmic reticulum quality control and sterol regulation.** *Mol Cell Biol* 2001, **21**:4276-4291.
This study demonstrates that glycerol stabilizes Hmg2p but does not affect the HRD pathway nor the generation of signals that programme Hmg2p degradation. Thus, the Hmg2p molecule behaves like a misfolded protein in cellular conditions where degradation signals are elevated.

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

15. •• Ravid T, Doolman R, Avner R, Harats D, Roitelman J: **The ubiquitin proteasome pathway mediates the regulated degradation of mammalian 3-hydroxy-3-methylglutaryl-coenzyme A reductase.** *J Biol Chem* 2000, **275**:35840-35847.
This work demonstrates that regulated ubiquitination of HMG-CoA reductase is conserved across the billion-year gulf that separates mammals from yeast. The magnitude and regulators are quite similar.

16. Donoviel DB, Donoviel MS, Fan E, Hadjantonakis A, Bernstein A: **Cloning and characterization of Sel-1l, a murine homolog of the *C. elegans sel-1* gene.** *Mech Dev* 1998, **78**:203-207.

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

18. Biunno I, Bernard L, Dear P, Cattaneo M, Volorio S, Zannini L, Bankier A, Zollo M: **SEL1L, the human homolog of *C. elegans sel-1*: refined physical mapping, gene structure and identification of polymorphic markers.** *Hum Genet* 2000, **106**:227-235.

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

20. Plemper RK, Deak PM, Otto RT, Wolf DH: **Re-entering the translocon from the lumenal side of the endoplasmic reticulum. Studies on mutated carboxypeptidase yscY species.** *FEBS Lett* 1999, **443**:241-245.

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

This and related work clearly show that the HMG-CoA reductase degradation ligase has a broad role in ER quality control, and that the envelope of substrates transcends sequence similarity.

22. Plemper RK, Bordallo J, Deak PM, Taxis C, Hitt R, Wolf DH: **Genetic interactions of Hrd3p and Der3p/Hrd1p with Sec61p suggest a retro-translocation complex mediating protein transport for ER degradation**. *J Cell Sci* 1999, **112**:4123-4134.

23. Friedlander R, Jarosch E, Urban J, Volkwein C, Sommer T:
•• **A regulatory link between ER-associated protein degradation and the unfolded-protein response.** *Nat Cell Biol* 2000, **2**:379-384.

See annotation Travers *et al.* (2000) [27••].

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

25. Wilhovsky S, Gardner R, Hampton R: **HRD gene dependence of endoplasmic reticulum-associated degradation.** *Mol Biol Cell* 2000, **11**:1697-1708.

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

This paper, together with [46•] and [47•], delineates aspects of the surprising observation that ER-to-Golgi traffic must be intact for ER-associated degradation (ERAD) of some, but not all, degradation substrates. This work and that of Caldwell *et al.* (2001) [46•] posit transport models, and Taxis *et al.* (2002) [47•] suggests a less direct role for ER-to-Golgi traffic in the observed ERAD effects.

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

This paper and Friedlander *et al.* (2000) [23••] make the very important observation that the HRD ligase is required for normal cells to maintain an acceptable level of unfolded proteins in the ER, and that this machinery is upregulated as one tactic to manage increases in the burden of lumenal unfolded proteins.

28. Braun S, Matuschewski K, Rape M, Thoms S, Jentsch S: **Role of the**
• **ubiquitin-selective CDC48(UFD1/NPL4) chaperone (segregase) in ERAD of OLE1 and other substrates.** *EMBO J* 2002, **21**:615-621.

See annotation Bays *et al.* (2001) [11•].

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

See annotation Bays *et al.* (2001) [11•].

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

See annotation Bays *et al.* (2001) [11•].

31. Rape M, Hoppe T, Gorr I, Kalocay M, Richly H, Jentsch S:
• **Mobilization of processed, membrane-tethered SPT23 transcription factor by CDC48(UFD1/NPL4), a ubiquitin-selective chaperone.** *Cell* 2001, **107**:667-677.

See annotation Bays *et al.* (2001) [11•].

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

See annotation Bays *et al.* (2001) [11•].

33. Hitchcock AL, Krebber H, Frietze S, Lin A, Latterich M, Silver PA:
• **The conserved npl4 protein complex mediates proteasome-dependent membrane-bound transcription factor activation.** *Mol Biol Cell* 2001, **12**:3226-3241.

See annotation Bays *et al.* (2001) [11•].

34. Bays NW, Hampton RY: **Cdc48p/Npl4p/Ufd1p: stuck in the middle with Ub.** *Curr Biol* 2002, **12**:R366-R371.

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

36. Zhou M, Schekman R: **The engagement of Sec61p in the ER dislocation process.** *Mol Cell* 1999, **4**:925-934.

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

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

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

40. Swanson R, Locher M, Hochstrasser M: **A conserved ubiquitin**
•• **ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matalpha2 repressor degradation.** *Genes Dev* 2001, **15**:2660-2674.

This study identifies an ER-anchored ubiquitin ligase, Doa10p, which is completely distinct from Hrd1p/Der3p; demonstrating that there are multiple ER-associated degradation pathways working together to manage unfolded ER proteins.

41. Jiang J, Ballinger CA, Wu Y, Dai Q, Cyr DM, Hohfeld J, Patterson C:
• **CHIP is a U-box-dependent E3 ubiquitin ligase: identification of Hsc70 as a target for ubiquitylation.** *J Biol Chem* 2001, **276**:42938-42944.

This paper, with Connell *et al.* (2001) [42•] and Murata *et al.* (2001) [43•], show that the U-box protein CHIP can serves as an adaptor to assemble a chaperone-based quality control ubiquitin ligase, allowing specific recognition of misfolded proteins that bind to the recruited chaperone.

42. Connell P, Ballinger CA, Jiang J, Wu Y, Thompson LJ, Hohfeld J,
• Patterson C: **The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins.** *Nat Cell Biol* 2001, **3**:93-96.

See annotation Jiang *et al.* (2001) [41•].

43. Murata S, Minami Y, Minami M, Chiba T, Tanaka K: **CHIP is a**
• **chaperone-dependent E3 ligase that ubiquitylates unfolded protein.** *EMBO Rep* 2001, **2**:1133-1138.

See annotation Jiang *et al.* (2001) [41•].

44. Meacham GC, Patterson C, Zhang W, Younger JM, Cyr DM:
•• **The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation.** *Nat Cell Biol* 2001, **3**:100-105.

This is a particularly relevant CHIP paper for this review. It demonstrates a possible involvement of an 'adaptor ligase' in the degradation of the misfolded ER-membrane protein CFTR ΔF508.

45. Ponting CP: **Proteins of the endoplasmic-reticulum-associated degradation pathway: domain detection and function prediction.** *Biochem J* 2000, **351**:527-535.

46. Caldwell SR, Hill KJ, Cooper AA: **Degradation of endoplasmic**
• **reticulum (ER) quality control substrates requires transport between the ER and Golgi.** *J Biol Chem* 2001, **276**:23296-23303.

See annotation Vashist *et al.* (2001) [26•].

47. Taxis C, Vogel F, Wolf D: **ER–Golgi-traffic is a prerequisite for**
• **efficient ER degradation.** *Mol Biol Cell* 2002, in press.

See annotation Vashist *et al.* (2001) [26•].

48. Gardner RG, Hampton RY: **A highly conserved signal controls degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in eukaryotes.** *J Biol Chem* 1999, **274**:31671-31678.

49. Cronin SR, Khoury A, Ferry DK, Hampton RY: **Regulation of HMG-CoA reductase degradation requires the P-type ATPase Cod1p/Spf1p.** *J Cell Biol* 2000, **148**:915-924.

50. Cronin S, Rao R, Hampton RY: **Cod1p/Spf1 is an ER P-type ATPase required for ER function and calcium homeostasis.** *J Cell Biol* 2002, in press.

51. Gardner RG, Shan H, Matsuda SP, Hampton RY: **An oxysterol-derived positive signal for 3-hydroxy-3-methylglutaryl-CoA reductase degradation in yeast.** *J Biol Chem* 2001, **276**:8681-8694.

52. Meigs TE, Simoni RD: **Farnesol as a regulator of HMG-CoA reductase degradation: characterization and role of farnesyl pyrophosphatase.** *Arch Biochem Biophys* 1997, **345**:1-9.

53. Correll CC, Ng L, Edwards PA: **Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase.** *J Biol Chem* 1994, **269**:17390-17393.

54. Gardner RG, Hampton RY: **A 'distributed degron' allows regulated entry into the ER degradation pathway.** *EMBO J* 1999, **18**:5994-6004.

55. Sato S, Ward CL, Krouse ME, Wine JJ, Kopito RR: **Glycerol reverses the misfolding phenotype of the most common Cystic fibrosis mutation.** *J Biol Chem* 1996, **271**:635-638.

56. Brown CR, Hong-Brown LQ, Biwersi J, Verkman AS, Welch WJ: **Chemical chaperones correct the mutant phenotype of the delta F508 cystic fibrosis transmembrane conductance regulator protein.** *Cell Stress Chaperones* 1996, **1**:117-125.

57. Welch WJ, Brown CR: **Influence of molecular and chemical chaperones on protein folding.** *Cell Stress Chaperones* 1996, **1**:109-115.

58. Cheng HH, Xu L, Kumagai H, Simoni RD: **Oligomerization state influences the degradation rate of 3-hydroxy 3-methylglutaryl-CoA reductase.** *J Biol Chem* 1999, **274**:17171-17178.

59. Nohturfft A, Yabe D, Goldstein JL, Brown MS, Espenshade PJ: **Regulated step in cholesterol feedback localized to budding of SCAP from ER membranes.** *Cell* 2000, **102**:315-323.