The Role of Multiubiquitination in Dislocation and Degradation of the \( \alpha \) Subunit of the T Cell Antigen Receptor

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Unassembled \( \alpha \) subunits of the T cell receptor (TCR\( \alpha \)) are degraded by proteasomes following their dislocation from the endoplasmic reticulum membrane. We previously demonstrated that a variant of TCR\( \alpha \) lacking lysines (K\( \alpha \R) is degraded by this pathway with kinetics indistinguishable from those of the wild type protein (Yu, H., Kaung, G., Kobayashi, S., and Kopito, R. R. (1997) J. Biol. Chem. 272, 20800–20804), demonstrating that ubiquitination on lysines is not required for TCR\( \alpha \) degradation by the proteasome. Here, we show that dislocation and degradation of TCR\( \alpha \) and K\( \alpha \R are suppressed by dominant negative ubiquitin coexpression and by mutations in the ubiquitin activating enzyme, indicating that their degradation requires a functional ubiquitin pathway. A cytoplasmic TCR\( \alpha \) variant that mimics a dislocated degradation intermediate was degraded 5 times more rapidly than full-length TCR\( \alpha \), suggesting that dislocation from the endoplasmic reticulum membrane is the rate-limiting step in TCR\( \alpha \) degradation. We conclude that ubiquitination is required both for dislocation and for targeting TCR\( \alpha \) chains to the proteasome.

The ER is the site of biogenesis of integral membrane and secretory proteins in eukaryotic cells and, as such, provides an oxidizing environment, specialized molecular chaperones, and enzymes that facilitate the folding and assembly of these proteins. Nascent polypeptide chains destined for this compartment are translated on membrane-bound cytoplasmic ribosomes and targeted by signal sequences for translocation across or integration into the ER membrane (reviewed in Ref. 1). The translocation machinery, which minimally includes signal recognition particle, signal recognition particle receptor, and the SEC61p translocon, couples the synthesis of membrane and secretory proteins to their translocation across or integration into the ER membrane (1, 2). Such coupling is necessary to ensure efficient signal sequence recognition (3) and to prevent aggregation within the reducing, aqueous environment of the cytoplasm (4). In mammalian cells, cotranslational delivery of nascent chains by direct docking of ribosomes to the translocation apparatus appears to be essential for efficient ER translocation of secretory as well as integral membrane proteins (5).

Recent studies have revealed that translocation across the ER membrane is reversible. For example, translocated, glycosylated major histocompatibility complex class I heavy chains in human cytomegalovirus-infected cells (6, 7) and unassembled \( \alpha \) chains of the T cell receptor (TCR\( \alpha \)) (8, 9) are destroyed by cytoplasmic proteasomes by a process requiring their “dislocation” across the ER membrane and deglycosylation by \( \alpha \)-glycanase. Misfolded soluble proteins like mutant carboxypeptidase Y (10) and prepro-\( \alpha \) factor (11) as well as polytopic integral membrane proteins like cystic fibrosis conductance regulator CFTR (12, 13), Pdr5p (14), and Sec61p (15) are also degraded by this pathway, indicating that proteasomes may be the major engine of “quality control”-linked, ER-associated degradation (ERAD; Ref. 16) (reviewed in Refs. 17–19).

Dislocation is necessary for ERAD because proteasomes are restricted to the cytoplasmic and nuclear compartments, while ER degradation substrates are initially present either on the luminal side of, or are integrated into, the ER membrane. Because many ERAD substrates are extremely prone to aggregate if exposed to cytosol (4, 8), it is likely that dislocation and degradation are tightly coupled processes. Such coupling could be achieved, by analogy to biosynthetic translocation, by direct transfer of substrates to proteasomes “docked” at the ER membrane. However, because dislocated, deglycosylated, and often multiubiquitinated forms of misfolded glycoproteins are found to accumulate in the cytoplasm following acute proteasome inhibition (6, 20, 21) or overexpression (4), the coupling between dislocation and proteolysis cannot be strictly obligatory. Although recent genetic and biochemical studies suggest a role for the translocation machinery, including BIP, Sec61p, and Sec63p (22, 23), in the dislocation of some misfolded ER-derived proteins, how such substrates are recognized and delivered to the dislocation machinery and the proteasome is unknown.

Multiubiquitination is a well established signal that targets cytoplasmic proteins to proteasomes for degradation (for a comprehensive review, see Ref. 24). A role for multiubiquitination in the degradation of ER-derived proteins is less clear, however, because many of these substrates are topologically separated by the ER membrane from the Ub-conjugating machinery in the cytoplasm (24). Accumulation of multiubiquitinated ER-derived substrates following acute proteasome inhibition (4, 20, 25) could result from uncoupling of proteolysis and dislocation and, therefore, does not necessarily demonstrate that ubiquitin conjugates of these proteins are true intermediates. On the other hand, many ER-derived substrates are stabilized by mutant Ub-activating (12, 25) or -conjugating enzymes (10, 15, 26) or by “dominant negative” mutations in Ub chain elongation (12, 15), strongly suggesting a role for the Ub pathway in ERAD. Such mutant studies cannot, however, discriminate between a role for direct ubiquitin conjugation to the degradation substrate or an indirect signaling role for Ub. The studies
in this paper address the role of the ubiquitin pathway in the coupling of dislocation of an integral membrane protein, TCRα, to its degradation by cytoplasmic proteasomes.

TCRα is a 38-kDa type I glycoprotein composed of extracellular immunoglobulin-like domains, a single transmembrane domain, and a short (5-amino acid) cytoplasmic tail (27). Unassembled TCRα chains are unstable and are rapidly (t1/2 = 45–60 min) degraded (28, 29). Although it is well established that TCRα degradation requires dislocation to cytoplasmic proteasomes (8, 9), the role for ubiquitination in this process has been controversial. Multiubiquitinated forms of TCRα have been detected in some (20) but not all (8, 9) TCRα-expressing cells following exposure to proteasome inhibitors. We previously reported that the kinetics of dislocation and degradation of a lysineless variant of TCRα (KaR) were indistinguishable from those of the wild type protein (8), demonstrating that ubiquitination of TCRα on ε-amino groups is not required for its dislocation or its degradation by proteasomes. However, those studies did not determine whether ubiquitination could contribute to TCRα degradation directly, by conjugation to a nonlysine residue, or indirectly, by conjugation to unidentified proteins.

In the present study, we have employed alternative strategies to investigate the role of ubiquitin in the degradation of TCRα. Surprisingly, our data show that degradation of both TCRα and the lysineless variant, KaR, requires a functional ubiquitination apparatus. We also show that ubiquitination is required for dislocation of TCRα from the ER membrane, although no ubiquitinatable residues of this integral membrane protein are initially accessible to the cytoplasmic ubiquitination machinery. Finally, we present data suggesting that dislocation from the ER membrane is the rate-limiting step in TCRα degradation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—E36 and ts20 cells were maintained in RPMI medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 30 °C and 5% CO2. HEK cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, penicillin, and streptomycin at 37 °C and 5% CO2. The cells were transiently transfected by calcium phosphate precipitation as described above.

**Radioiodination of Ubiquitin and Cell-free Translation**—Bovine ubiquitin (Sigma) was radioiodinated using 1 mCi of [125I]NaI (17 Ci/mg, NEN Life Science Products) and IODO-GEN beads (Pierce). 1-Labeled Ub at 1–2 × 10^6 cpm/ml was purified by gel filtration. For in vitro ubiquitination, TCRα-HA and KaR-HA were each used to program 150-μl coupled transcription and translation reactions with rabbit reticulocyte lysate (TT-TNT; Promega) in the presence of [125I]labeled Ub (1 × 10^6 cpm/μl) for 90 min at 30 °C. TCRα-HA and KaR-HA were immunoprecipitated with monoclonal anti-HA antibody 12CA5 (Roche Molecular Biochemicals) and 50 μl of Gammabind Plus Sepharose beads. [125I]Ub-labeled TCRα-HA and KaR-HA were quantified by PhosphoImager analysis (Molecular Dynamics). For the control sample, empty pCDNA3.1 vector was used in place of the TCRα-HA and KaR-HA cDNA to program the translation reactions. For immunoblotting of in vitro translated TCRα-HA and KaR-HA, 125I-labeled Ub was omitted from the coupled transcription and translation reactions. The translation products were immunoprecipitated with 12CA5 and immunoblotted with rabbit anti-HA antibody HA.11 (Babco).

**RESULTS**

**Degradation and Ubiquitination of a Lysineless TCRα Variant**—In this study, we sought to evaluate the role of Ub in the degradation of the lysineless TCRα variant, KaR. Because the internal epitope recognized by anti-TCRα antibodies may be obscured by ubiquitination of internal lysine residues, we performed pulse-chase analysis on human embryonic kidney 293 (HEK) cells expressing epitope-tagged variants, TCRα-HA and KaR-HA (Fig. 1A). Both proteins were rapidly (t1/2 ~ 30 min) degraded during the chase with indistinguishable kinetics. These kinetics are slightly faster than those observed for the native TCRα (8, 28, 29), suggesting that the presence of a HA epitope further destabilizes this protein. Nonetheless, the data establish that TCRα degradation does not require ubiquitination of Lys residues (8) and that the HA epitope tag does not interfere with targeting of these proteins to the proteasome.

To determine the extent to which elimination of lysine residues reduced the capacity of TCRα to be ubiquitinated, TCRα-HA and KaR-HA were used to program cell-free transcription and translation reactions in rabbit reticulocyte lysate supplemented with [125I]labeled ubiquitin. Total TCRα-HA and KaR-HA synthesis and [125I]-labeled Ub conjugates thereof were assessed by anti-HA immunoblotting or by autoradiography, respectively, of HA-immunoprecipitated translation products (Fig. 1B). Although both proteins were synthesized and immunoprecipitated with equivalent efficiency (Fig. 1B; lanes 1–3),
substitution of the 11 Lys residues in TCRα-HA with Arg reduced the level of 125I-labeled Ub conjugation by 91% (Fig. 1B, lanes 4–6). The residual ubiquitination of KoR-HA in this assay suggests that Ub must be conjugated to nonlysine sites, most likely the N-terminal α-amino group, as was recently shown for the transcription factor MyoD (33). These data show that the effect of eliminating Lys residues on the kinetics of TCRα degradation does not correlate with the effect of this mutation on the capacity of the protein to become ubiquitinated in vitro. Together, these results suggest either that ubiquitination is not involved in the degradation of TCRα or that ubiquitination at nonlysine residue(s) is sufficient to target the protein to the proteasome.

Ubiquitination Is Required for Degradation of Wild Type TCRα and a Lysineless Variant—Since the capacity of TCRα-HA and KoR-HA to become multiubiquitinated fails to correlate with their degradation kinetics, we used an alternative method to assess the role of the ubiquitin pathway in TCRα and KoR degradation. Individual ubiquitin moieties within multiubiquitin chains are linked by isopeptide bonds between the carboxyl group of the terminal glycine residue of one ubiquitin and the ε-amino group of a lysine residue, usually Lys48, in the next monomer (34, 35). Expression of mutant Ub with Lys substituted by Arg (K48R) exerts a dominant-negative, chain-terminating effect on multiubiquitin chains and on the degradation of cytoplasmic (35, 36) and ER-derived substrates (12, 15) by the ubiquitin-proteasome pathway. Pulse-chase analysis (Fig. 2A) revealed that coexpression of K48R-ubiquitin (HM-K48R-Ub) (12) strongly stabilized wild type TCRα, extending its half-life to nearly 3 h, while coexpression of wild type Ub (HM-Ub) had no detectable effect on TCRα degradation. Because the effect of K48R coexpression on targeting substrates to the proteasome is dependent on the position in a multiubiquitin chain at which the mutant Ub is incorporated, and because the expressed mutant Ub must compete with the large pool of wild type Ub in the cell, the effect of K48R coexpression on degradation is always significantly less than 100%. Nonetheless, we found that coexpression of K48R-Ub inhibited the degradation of the lysineless construct KoR to a similar extent (Fig. 2B) as wild type TCRα, indicating that, even in the absence of ε-amino groups, multiubiquitination machinery is in some way required for TCRα degradation.

To confirm a role for ubiquitination in TCRα degradation, we expressed TCRα in ts20 cells that harbor a thermolabile allele of the ubiquitin-activating enzyme (E1) (Fig. 3A). At the restrictive temperature (40 °C), ubiquitination and, consequently, ubiquitin-dependent protein degradation by these cells are inhibited (37). Degradation of TCRα in these cells was blocked by N-acetyl-leucyl-leucyl-norleucinal and lactacystin, indicating that, as in HEK cells, it is mediated by the proteasome (data not shown). Shifting to the nonpermissive temperature of 40 °C profoundly inhibited TCRα degradation in ts20 cells (Fig. 3A) but not in the parental E36 cells (Fig. 3B). Together, these data unambiguously support a role for Ub conjugation in the degradation of both TCRα and KoR. However, because TCRα has no ubiquitinatable residues initially accessible to the cytoplasm, direct ubiquitination of TCRα cannot precede its dislocation. Moreover, a functional role for co-dislocaional ubiquitination of TCRα is unlikely, because KoR is degraded at the same rate as TCRα, although it has no free internal amino groups.

Kinetic Coupling between Dislocation and Degradation of TCRα—The preceding discussion implies that the only way for direct ubiquitination of TCRα to contribute to its degradation is if the protein is first dislocated to the cytoplasm and then ubiquitinated. In this manner, ubiquitination of TCRα could serve to functionally couple dislocation and degradation if and only if the rate of TCRα ubiquitination is fast compared with the rate of its dislocation from the membrane. The observation that deglycosylated forms of TCRα accumulate in the cytoplasm following chronic exposure of cells to proteasome inhibitors (8, 9) is consistent with such a model. To determine whether cells possess the capacity to recognize and rapidly degrade dislocated TCRα molecules, we experimentally uncou-
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Degradation of TCRα and Kα Requires Multiquitination. HEK cells were co-transfected with TCRα (A) or Kα (B) together with an empty vector (Vector), His6Myc-tagged wild type ubiquitin (HM-Ub), or His6Myc-tagged K48R-ubiquitin (HM-K48R-Ub). The cells were pulse-labeled with [35S]Met/Cys and chased for the indicated times. TCRα and Kα were immunoprecipitated at each point by monoclonal antibody H28-710 and resolved by SDS-PAGE. Band intensities were quantified by densitometry after fluorography or by PhosphorImager analysis and represented as a percentage of the signal at 0 min. Individual data points correspond to mean values obtained from three separate experiments. S.D. values are indicated by the error bars; n = 3.

Degradation of TCRα was more rapid than the full-length TCRα, with a 4.5-fold slower rate observed for the full-length TCRα. Thus, although lysine residues are not required for degradation of TCRα dislocated from the membrane, they are required for the extremely rapid degradation rate observed for this hypothetical cytoplasmic TCRα intermediate. The kinetic rates of TCRα and ΔssKα degradation were evaluated by pulse-chase analysis (Fig. 4). Following a 10-min pulse labeling with [35S]Met, the majority of both proteins was soluble in Nonidet P-40 and deoxycholate. A small fraction of these proteins was chased into a detergent-insoluble pool. Strikingly, subsequent chase revealed that ΔssTCRα was degraded 5 times more rapidly than the full-length protein (t1/2 ~ 10 min) (Fig. 4, A and E). The rapid degradation was strongly inhibited by clasto-lactacycin β-lactone.
clastolactacin β-lactone (38) (Fig. 4, B and E), indicating that it was mediated by the proteasome. However, since this inhibitor did not completely block the disappearance of the proteins, it is possible that other proteases may also participate in ΔssTCRα degradation. Alternatively, this disappearance may reflect the sequestration of these proteins into an aggresome-like (4) pool that is inaccessible to immunoprecipitation by our antibodies. Indeed, following prolonged incubation with lactacin, ΔssTCRα can be observed in aggresomes (data not shown).

These observations suggest that Ub conjugation to amino groups on dislocated, cytoplasmic TCRα may contribute to kinetic coupling between dislocation and degradation. To assess the role of multiubiquitination in this process, we evaluated the effects of K48R-Ub co-expression on the stability of ΔssTCRα (Fig. 5A) and ΔssKoR (Fig. 5B). The data show that K48R-Ub inhibited ΔssTCRα degradation to a modest extent, suggesting that the extreme instability of this protein is at least in part due to Ub-dependent processes. By contrast, K48R-Ub coexpression led to dramatic stabilization of ΔssKoR, extending the t1/2 almost 10-fold to over 1.5 h. It is likely that, in the absence of ε-amino groups, the α-N terminus of ΔssKoR serves as a functional multiubiquitin conjugation site, as recently shown for a short lived cytoplasmic protein, MyoD (33). To inhibit multiubiquitin chain formation, K48R-Ub molecules must compete with endogenous wild type Ub for conjugation. The greater extent to which K48R-Ub inhibited ΔssKoR degradation is most likely, therefore, a consequence of the fact that there are 12 times as many potential ubiquitination sites on ΔssKoR as on the corresponding lysineless variant.

**Ubiquitination Is Required for TCRα Dislocation**—The degradation by cytoplasmic proteasomes of ER-derived proteins involves at least three distinct steps: substrate recognition, dislocation across the ER membrane, and delivery to proteasomes for degradation. For proteins with large cytoplasmic domains, like CFTR, Ub could participate in and serve to couple all three steps (39). However, for proteins that lack any cytoplasmically exposed ubiquitatable groups, like TCRα, direct ubiquitination of substrates cannot contribute to the latter two steps. The data presented above suggest such a role. Although Ub cannot participate in the direct tagging of dislocation substrates in the ER lumen, it is possible that Ub could participate indirectly in the dislocation process. We have previously reported that dislocation of TCRα is accompanied by progressive removal of N-linked high mannose glycans, leading to an approximately 10-kDa decrease in Mₐ, and by increasing sensitivity to degradation by exogenous proteases (8). We therefore assessed the effect of K48R-Ub coexpression on the mobility and protease accessibility of TCRα (Fig. 6). If multiubiquitination is required for degradation but not dislocation of TCRα, then K48R-Ub coexpression should have a qualitatively similar effect to lactacin, causing newly synthesized fully glycosylated, protease-resistant TCRα chains to accumulate in a glycosylated, protease-accessible form. If multiubiquitination is required for dislocation, then the undegraded TCRα chains should remain protease-resistant and glycosylated. The data in Fig. 6 indicate that, although the K48R coexpression clearly inhibited TCRα degradation, extending the t1/2 to nearly 3 h, none of the labeled undegraded protein became dislocated. These data suggest that multiubiquitination is required for dislocation of TCRα from the ER.

**DISCUSSION**

Proteasomes in the cytoplasmic compartment of eukaryotic cells constitute a major pathway for degradation of ER-derived proteins. Because the substrates are physically separated by the ER membrane from the proteases that degrade them, ERAD substrates must be translocated across this barrier through a process that has been termed “dislocation” (6). In this paper, we have investigated the mechanism by which the dislocation of a model ERAD substrate, TCRα, is coupled to its degradation by cytoplasmic proteasomes. Our data suggest that dislocation is rate-limiting for ERAD and reveal that multiubiquitination is required both for dislocation and for kinetic coupling of dislocation to degradation.

Although the role of proteasomes in ERAD is well established, the mechanism by which substrates of this pathway are recognized and delivered to proteasomes is unknown. A role for Ub is suggested from studies showing that transmembrane and luminal ERAD substrates are stabilized by mutations in ubiquitin-conjugating enzymes (10, 14, 26, 40) and by coexpression of dominant negative Ub mutants (12, 15). However, all known components of the Ub conjugation pathway are either cytosolic (41, 42) or attached to the cytosolic face of the ER membrane (42, 43). Moreover, these studies failed to discriminate between direct (i.e. Ub conjugation to the ERAD substrate) and indirect (i.e. in trans Ub conjugation to components other than substrate) ubiquitination. Some ERAD substrates accumulate as cytoplasmic high molecular weight multiubiquitin conjugates in cells with genetically (44) or pharmacologically enfeebled (12) proteasomes. However, *bona fide* ubiquitinated intermediates have not been observed in cells that are unperturbed by
either proteasome inhibition or substrate overexpression. Functional inhibition of ubiquitin conjugation machinery demonstrated a requirement for ubiquitin-conjugating enzymes and the ubiquitin-activating enzyme E1 in both the dislocation and the degradation of luminal proteins, mutant carboxypeptidase Y (10) and truncated ribophorin I (25). Because mutant carboxypeptidase Y and truncated ribophorin I are completely translocated into the ER lumen prior to their degradation by proteasomes, direct ubiquitination can only occur during or after dislocation, possibly by direct, co-dislocational ubiquitination of internal lysine ε-amino groups acting as a "Brownian ratchet" to drive dislocation. On the other hand, it is also possible that direct ubiquitination is not required for ERAD. Dislocation of integral membrane ERAD substrates is inhibited by proteasome mutation (14, 40) and by proteasome inhibitors (8), suggesting that dislocation is tightly coupled to degradation and raising the possibility that proteasomes may directly "extract" substrates from the ER membrane in the absence of direct ubiquitination.

In this study, we have sought to discriminate between these models by investigating the role of Ub in dislocation and degradation of KαR, a lysineless variant of a type I integral membrane glycoprotein, TCRα. Both the kinetics and proteasome dependence of KαR degradation are indistinguishable from those of its wild type counterpart, TCRα, which contains 11 lysine residues, although in the present work, we show that the capacity for the lysineless variant to become ubiquitinated in vitro is reduced by more than 90%. Because KαR has no internal lysine residues, these data argue strongly against a Brownian ratchet mechanism for TCRα degradation. Our data reveal that, remarkably, degradation of both KαR and TCRα is suppressed to the same extent by coexpression of a dominant negative Ub construct (K48R-Ub), suggesting a role for Ub that is independent of modification of substrate ε-amino groups. Moreover, K48R coexpression indicates that Ub is actually required for dislocation of TCRα. Although Ub can, in theory, be conjugated to other sites including Ser, Thr, or Cys residues, via ester or thiol ester bonds, these conjugates are likely to be extremely labile and have never been demonstrated. Alternatively, ubiquitination of the α-amino group is both necessary and sufficient for targeting of at least one cytoplasmic substrate to the proteasome (33). Because an efficient in vitro system reconstituting membrane dislocation is not yet available, it is not possible to directly test the role of the N-terminal α-amino group in TCRα degradation.

We therefore propose two alternative models to explain the requirement for multibiligation in dislocation of TCRα (Fig. 7). Since TCRα is a type I protein that is targeted for translocation across the ER membrane by means of a conventional N-terminal signal sequence, its N terminus must be, prior to dislocation, luminal. If direct ubiquitination is required for KαR degradation, the N terminus must exit the dislocation pore first, i.e. before the rest of the protein. Conceivably, cooperation between luminal chaperones, the translocon and KαR could initiate the formation of such an intermediate as depicted in Fig. 7A. The only alternative explanation for our data is that Ub is required indirectly for dislocation (Fig. 7B). In this model, the presence or persistence of an unassembled or misfolded membrane protein in the translocon could signal ubiquitination of one or more translocon-associated polypeptides. Interestingly, ubiquitination of the ER chaperone, calnexin, was reported to be induced during the dislocation and degradation of a misfolded luminal protein, mutant α1-antitrypsin (45). According to this model, Ub could contribute an in trans signaling role, perhaps recruiting proteasomes to the dislocation site. Future studies in which dislocation is reconstituted in a cell-free system will be needed to ultimately clarify the role of Ub in membrane dislocation. Finally, we employed a TCRα variant lacking a signal sequence (ΔssTCRα) to determine whether recognition by the cytoplasmic Ub/proteasome pathway is fast enough to couple the dislocation of TCRα to its degradation. The rapid rate of ΔssTCRα degradation suggests that the rate-limiting step for the degradation of the full-length protein is its dislocation from the ER. Alternatively, these data do not exclude the possibility that the conformation (and susceptibility to proteasomal degradation) of signal sequenceless TCRα may differ depending on whether it entered the cytoplasm directly following translation from cytoplasmic ribosomes or indirectly via dislocation from the ER. Although our data are not inconsistent with the possibility that dislocation is mechanistically coupled to proteolysis, as suggested by Mayer et al. (40), they indicate that kinetics alone are sufficient to account for the tight coupling observed between dislocation and degradation.

In conclusion, our data suggest a role for Ub both in dislocation of an unassembled membrane protein from the ER and in
the coupling of dislocation to degradation by the proteasome. It is likely that the role of Ub and of other, yet unidentified membrane and cytoplasmic components in ERAD will depend on the topology of the particular substrate and on the nature of the degradation signals therein.

Acknowledgments—We are grateful to Dr. John Moorehead (University of Colorado Health Science Center) for generously reading of the manuscript. Dr. Marina Gelman for many helpful discussions and critical reading of the manuscript.

REFERENCES
1. Corsi, A. K., and Schekman, R. (1996) J. Biol. Chem. 271, 30329–30332
2. Rapoport, T. A., Jungnickel, B., and Klatz, U. (1996) Annu. Rev. Biochem. 65, 271–303
3. Rothman, J. E., and Lodish, H. F. (1977) Nature 269, 775–780
4. Johnston, J. A., Ward, C. L., and Kopito, R. R. (1998) J. Cell Biol. 143, 1883–1898
5. Hegde, R. S., and Lingappa, V. R. (1997) Cell 91, 575–582
6. Wiertz, E. J., Jones, T. R., Sun, L., Bogyo, M., Geuze, H. J., and Ploegh, H. L. (1996) Cell 84, 769–779
7. Wiertz, E. J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T. R., Rapoport, T. A., and Ploegh, H. L. (1996) Nature 384, 432–438
8. Yu, H., Kaung, G., Kobayashi, S., and Kopito, R. R. (1997) J. Biol. Chem. 272, 20800–20804
9. Huppa, J. B., and Ploegh, H. L. (1997) Immunity 7, 113–122
10. Miller, D. M., Lippincott-Schwartz, J., Weissman, A. M., and Klausner, R. D. (1989) Science 243, 1576–1583
11. Werner, E. D., Brodsky, J. L., and McCracken, A. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 13797–13801
12. Ward, C. L., Omura, S., and Kopito, R. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 94, 1896–1901
13. Plemper, R. K., Biesemler, S., Bordallo, J., Sommer, T., and Wolf, D. H. (1997) J. Cell Biol. 138, 891–895
14. Pilet, M., Schekman, R., and Romisch, K. (1997) EMBO J. 16, 4540–4548
15. Herschko, A., and Ciechanover, A. (1998) Annu. Rev. Biochem. 67, 425–479
16. de Virgilio, M., Weninger, H., and Ives, N. E. (1998) J. Biol. Chem. 273, 9734–9743
17. Hampton, R. Y., and Bhakta, H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12944–12948
18. Bonifacino, J. S., Suzuki, C. K., Lippincott-Schwartz, J., Weissman, A. M., and Klausner, R. D. (1998) Cell 54, 209–220
19. Yu, H., Kaung, G., Kobayashi, S., and Kopito, R. R. (1997) J. Biol. Chem. 272, 20800–20804
20. Yang, M., Omura, S., Bonifacino, J. S., and Weissman, A. M. (1998) J. Exp. Med. 187, 835–846
21. Hughes, E. A., Hammond, C., and Cresswell, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1896–1901
22. Plemper, R. K., Biesemler, S., Bordallo, J., Sommer, T., and Wolf, D. H. (1997) Nature 388, 891–895
23. Pilen, M., Schekman, R., and Romisch, K. (1997) EMBO J. 16, 4540–4548
24. Hershko, A., and Ciechanover, A. (1998) Annu. Rev. Biochem. 67, 425–479
25. de Virgilio, M., Weninger, H., and Ives, N. E. (1998) J. Biol. Chem. 273, 9734–9743
26. Hampton, R. Y., and Bhakta, H. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12944–12948
27. Chien, Y., Becker, D. M., Lindsten, T., Okamura, M., Cohen, D. L., and Davis, M. M. (1984) Nature 312, 31–35
28. Lippincott-Schwartz, J., Bonifacino, J. S., Yuan, L. C., and Klausner, R. D. (1988) Cell 54, 209–220
29. Bonifacino, J. S., Suzuki, C. K., Lippincott-Schwartz, J., Weissman, A. M., and Klausner, R. D. (1989) J. Cell Biol. 109, 73–83
30. Ward, C. L., and Kopito, R. R. (1994) J. Biol. Chem. 269, 25710–25718
31. Saito, T., Weiss, A., Miller, J., Norcross, M. A., and Germain, R. N. (1987) Nature 325, 125–130
32. Becker, M. L., Near, H., Mudgett-Hunter, M., Margolies, M. N., Kuo, R. T., Kaye, J., and Hedrick, S. M. (1989) Cell 58, 911–921
33. Breitschopf, K., Bengal, E., and Ciechanover, A. (1998) EMBO J. 17, 5984–5993
34. Hershko, A., Ciechanover, A., Heller, H., Haas, A. L., and Rose, I. A. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1783–1786
35. Chau, V., Tobias, J. W., Bachmair, A., Marriott, D., Ecker, D. J., Gonda, D. K., and Varshavsky, A. (1989) Science 243, 1576–1583
36. Finley, D., Sadis, S., Monia, B. P., Boucher, P., Ecker, D. J., Crooke, S. T., and Chau, V. (1994) Mol. Cell. Biol. 14, 5501–5509
37. Kulka, R. G., Raboy, B., Schuster, R., Parag, H. A., Diamond, G., Ciechanover, A., and Varshavsky, A. (1988) Science 243, 1576–1583
38. Dick, L. R., Cruikshank, A. A., Grenier, L., Melandri, F. D., Nunes, S. L., and Stein, R. L. (1996) J. Biol. Chem. 271, 7273–7276
39. Xiong, X., Chong, E., and Skach, W. R. (1999) J. Biol. Chem. 274, 2029–2044
40. Mayer, T. U., Braun, T., and Jentsch, S. (1998) EMBO J. 17, 3251–3257
41. Ciechanover, A. (1998) EMBO J. 17, 7151–7160
42. Sommer, T., and Jentsch, S. (1993) Nature 365, 176–179
43. Biederer, T., Volkwein, C., and Sommer, T. (1997) Science 278, 1806–1809
44. Hampton, R. Y., Gardner, R. G., and Rine, J. (1996) Mol. Biol. Cell 7, 2029–2044
45. Qu, D., Teckman, J. H., Omura, S., and Perlmutter, D. H. (1996) J. Biol. Chem. 271, 27291–27295