Enhancement of Endoplasmic Reticulum (ER) Degradation of Misfolded Null Hong Kong α₁-Antitrypsin by Human ER Mannosidase I*

Nobuko Hosokawa‡§, Linda O. Tremblay||, Zhipeng You†, Annette Herscovics‡, Ikuo Wada***‡‡, and Kazuhiro Nagata$$$¶¶

From the ‡Department of Molecular and Cellular Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8397, Japan, ||Core Research for Ecological Science and Technology, Japan Science and Technology Corporation, Saitama 332-0012, Japan, |||McGill Cancer Centre, McGill University, Montréal, Québec H3G 1Y6, Canada, and §§Department of Biochemistry, Sapporo Medical University School of Medicine, Sapporo 060-8556, Japan.

© 2003 by The American Society for Biochemistry and Molecular Biology, Inc.

Received for publication, April 2, 2003, and in revised form, May 3, 2003
Published, JBC Papers in Press, May 6, 2003, DOI 10.1074/jbc.M303395200

Misfolded glycoproteins synthesized in the endoplasmic reticulum (ER) are degraded by cytoplasmic proteasomes, a mechanism known as ERAD (ER-associated degradation). In the present study, we demonstrate that ERAD of the misfolded genetic variant-null Hong Kong α₁-antitrypsin is enhanced by overexpression of the ER processing α₁,2-mannosidase (ER ManI) in HEK 293 cells, indicating the importance of ER ManI in glycoprotein quality control. We showed previously that EDEM, an enzymatically inactive mannose homolog, interacts with misfolded α₁-antitrypsin and accelerates its degradation (Hosokawa, N., Wada, I., Hasegawa, K., Yorihizu, T., Tremblay, L. O., Herscovics, A., and Nagata, K. (2001) EMBO Rep. 2, 415–422). Herein we demonstrate a combined effect of ER ManI and EDEM on ERAD of misfolded α₁-antitrypsin. We also show that misfolded α₁-antitrypsin NHK contains labeled Glc₃Man₆GlcNAc and Man₅₋₇GlcNAc released by endo-β-N-acetylglucosaminidase H in pulse-chase experiments with [2-³H]mannose. Overexpression of ER ManI greatly increases the formation of Man₅GlcNAc, induces the formation of Glc₃Man₆GlcNAc and increases trimming to Man₅₋₇GlcNAc. We propose a model whereby the misfolded glycoprotein interacts with ER ManI and with EDEM, before being recognized by downstream ERAD components. This detailed characterization of oligosaccharides associated with a misfolded glycoprotein raises the possibility that the carbohydrate recognition determinant triggering ERAD may not be restricted to Man₅GlcNAc isomer B as previous studies have suggested.

The synthesis of glycoproteins containing N-glycans begins in the endoplasmic reticulum (ER)* with the addition of the Glc₃Man₆GlcNAc2 precursor of N-linked glycans to nascent polypeptide chains. Subsequently, the ER lectin chaperones, calnexin or calreticulin, specifically bind to monoglycosylated N-glycans, promoting proper glycoprotein folding. This ER quality control process is mediated by glucosidases I and II that remove the glucose residues from Glc₃Man₆GlcNAc₂ and by UDP-glucose:glycoprotein glucosyltransferase that reglucosylates incompletely folded glycoproteins. Correctly folded glycoproteins exit the ER to their final destinations, whereas misfolded glycoproteins are readily degraded (for reviews see Refs. 1–4). Many terminally misfolded proteins in the ER are degraded by cytoplasmic proteasomes, a mechanism known as ERAD (for reviews see Refs. 5–7). Experimental evidence in yeast and mammalian cells suggests that ER α₁,2-mannosidase I (ER ManI) that primarily removes the middle branch mannose from Man₅GlcNAc₂ to form Man₄GlcNAc isomer B (ManSB) (8, 9) acts as a signal triggering ERAD of glycoproteins (10–12). Disruption of the ER α-mannosidase gene in yeast (10, 11) and inhibition of ER ManI in mammalian cells (13, 14) both prevent ERAD. We recently reported the molecular cloning of mouse EDEM (ER degradation enhancing α-mannosidase-like protein) and its involvement in glycoprotein ERAD. It was suggested that EDEM is a putative lectin, which most likely binds ManSB on misfolded α₁-antitrypsin and accelerates its degradation (15). Two groups also reported the yeast homolog of EDEM and its function in glycoprotein ERAD. We also demonstrated that ER ManI and EDEM are ER resident transmembrane proteins containing characteristic signature motifs of class I α₁,2-mannosidases (glycosylhydrolyase family 47). Despite this significant sequence similarity, EDEM lacks α₁,2-mannosidase activity with Man₅GlcNAc as substrate (15). In addition, these two proteins differ in their response to ER stress. EDEM is induced by various forms of ER stress, but ER ManI is not (15).

In this report, we show that overexpression of human ER ManI accelerates the degradation of the terminally misfolded α₁-antitrypsin genetic variant-null Hong Kong (A1AT NHK) (16). We also show that the combined effects of ER ManI and EDEM on the degradation of misfolded α₁-antitrypsin. Glycan analysis on misfolded NHK shows that overexpression of ER ManI greatly increases the formation of Man₅GlcNAc₂ and hancing α-mannosidase-like protein; ManSB, Man₅GlcNAc₂ isomer B; H923, human embryonic kidney 293; Endo-H, endo-β-N-acetylglucosaminidase H; DDE, Dulbecco’s modified Eagle’s medium; HA, hemagglutinin; PVDF, polyvinylidene difluoride.
**ER ManI Enhances Glycoprotein ERAD**

Glc₂Man₃GlcNAc₂ and also stimulates trimming of N-linked oligosaccharides to Man₃GlcNAc₂ in *vivo*. These experiments suggest that the misfolded glycoprotein interacts both with ER ManI and EDEM before being recognized by downstream ERAD components.

**EXPERIMENTAL PROCEDURES**

### Cell Culture and Transfection

Human HEK 293 cells were cultured in DMEM supplemented with 10% fetal bovine serum, and transfection of plasmids encoding human ER ManI, mouse EDEM, and human α1-antitrypsin-null Hong Kong were performed using FuGENE 6 transfection reagents (Roche Molecular Biochemicals) according to the protocol recommended by the manufacturer. Approximately 1.5 × 10⁶ cells were plated on poly-L-lysine-coated 3.5-cm dishes. After 24 h of plating, 0.5 µg of plasmid encoding ER ManI, EDEM, or pMH vector were mixed with 1 µg of plasmid encoding the α1-antitrypsin variant A1AT NHK for transfection, and cells were pulse-labeled and harvested 36 h post-transfection.

### Plasmid Construction

Human ER ManI cDNA was subcloned into the pMH vector (Roche Molecular Biochemicals) by PCR. The entire open reading frame (9) was amplified from cDNA using a sense primer containing a HindIII site and a Kozak sequence (5′-AAAAGACCCCTT CACCATGGTCGGTCCGAGGCGGAGG-3′) and an antisense primer containing a NotI site and omitting the stop codon (5′-AAA AAAAAACGGCGGCGGTCCGAGGCGGAGG-3′). The open reading frame amplicon was digested with HindIII and NotI and cloned into the HindIII/NotI sites of pMH in-frame with the C-terminal HA-tag. Mouse EDEM cDNA was tagged with HA at its C terminus in pCMV-Sport2 vector (Invitrogen) as described previously (15). The NHK variant was constructed as described elsewhere (15).

### Metabolic Labeling and Immunoprecipitation

Cells were preincubated in DMEM lacking methionine (Invitrogen) for 30 min and labeled with [3H]mannose for 30 min in DMEM supplemented with 0.02% [3H]mannose (specific activity 40 Ci/mmol) to label the NHK glycoprotein. The proteasome inhibitor lactacystin was purchased from Cappel (ICN Pharmaceuticals, Inc.), and purified IgG against the HA-epitope superfamily. Mutations in α1-antitrypsin cause emphysema or liver cirrhosis (23). The NHK genetic variant of A1AT misfolds terminally in the ER and is degraded by ERAD (12, 15).

### RESULTS

**Enhanced Degradation of A1AT NHK by Overexpression of ER ManI**—To examine the effect of ER mannosidase I on ERAD of misfolded glycoproteins, human ER ManI cDNA was transfected into HEK 293 cells along with the A1AT NHK. A1AT is a serum protein belonging to a serine protease inhibitor superfamily. Mutations in α1-antitrypsin cause emphysema or liver cirrhosis (23). The NHK genetic variant of A1AT misfolds terminally in the ER and is degraded by ERAD (12, 15).

Transfection of human ER ManI greatly enhanced the degradation of misfolded A1AT NHK in comparison with mock transfected cells (Fig. 1A, closed arrow, compare lanes 1–3 with lanes 4–6). NHK was not secreted into the medium of cells co-transfected with ER ManI (data not shown) nor of cells co-transfected with vector alone (15, 24). Quantitative analysis revealed that the t1/2 of transfected NHK in 293 cells was ~100 min. Co-transfection of ER ManI shortened the t1/2 of NHK to about 50 min (Fig. 1C).

NHK immunoprecipitated from cell lysates transfected with ER ManI migrated slightly faster on SDS-PAGE than that from mock transfected cells (Fig. 1A, closed arrow). Kifunsine inhibited the degradation of NHK in cells co-transfected with ER ManI (Fig. 2, A and C) and also resulted in a small decrease in NHK electrophoretic mobility, consistent with inhibition of ER α1,2-mannosidase activity (Fig. 2A, black arrow, compare lanes 1–3 with lanes 4–6). Therefore overexpression of ER ManI enhances ERAD of misfolded NHK by accelerating mannose trimming from its N-linked glycans. This conclusion was further supported by NHK oligosaccharide analyses described below.

Co-immunoprecipitation of ER ManI with NHK was detected in cells transfected with ER ManI (Fig. 1A, open arrow). Notably, the transfected ER ManI was degraded very rapidly (Fig. 1B, lanes 4–6, and see below). A broad uncharacterized protein band of ~170 kDa was co-immunoprecipitated with ER ManI.

**Antibodies**—Antiserum against α1-antitrypsin was purchased from Cappel (ICN Pharmaceuticals, Inc.), and purified IgG against the HA-tag was obtained from Santa Cruz Biotechnology, Inc. Human ER ManI antibody was produced by immunizing rabbits with a keyhole limpet hemocyanin-conjugated synthetic peptide (GRRDVEKPAADRHHLLR- PET). The antiserum was affinity-purified using the peptide. For the antibody against mouse EDEM, multiple antigenic peptide was synthesized using the following peptide sequence, DERRYSPLFKSHYMQRID.

Whole rabbit serum was used for the experiments.

**Western Blotting**—Nonidet P-40-soluble cell lysates or immunoprecipitates prepared as described above (see "Metabolic Labeling and Immunoprecipitation") were adjusted to 1 × Laemmli’s buffer containing 0.1% SDS and separated by 10% SDS-PAGE. Western blotting was performed on nitrocellulose membranes. Membranes were incubated with the monoclonal antibody against A1AT, and the immunoprecipitates were detected with the polyclonal antibody against A1AT, and the immune complexes were detected with alkaline phosphatase-conjugated goat anti-rabbit IgG and with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium as substrates.

**Characterization of Oligosaccharides**—The [3H]mannose-labeled NHK bands on the PVDF membranes were cut into small pieces. The pieces were first rinsed with methanol and then with 0.1% sodium citrate, pH 5.5, three times for 15 min at 37 °C, and the washes were dried. The membrane pieces were then incubated with Endo-H (New England Biolabs) in the same buffer at 37 °C for 48 h. Endo-H (1000 units) was added at 0, 12, and 36 h of incubation, and the membrane pieces were washed three times with water. The extracts and washes were combined, boiled for 3 min, and lyophilized. The labeled oligosaccharides completely released by this procedure were dissolved in water, mixed with standard [14C]labeled Glc₃Man₉GlcNAc₂ described by HPLC, and fractionated by HPLC at 4 °C. In some cases, an internal standard of [14C]-labeled Glc₂Man₂GlcNAc₂ was also used. This was prepared by treatment of labeled Glc₂Man₂GlcNAc₂ for 45 min at 37 °C with 0.2 µg of purified recombinant α1,2-mannosidase from *Saccharomyces cerevisiae* (22).
weight marker positions are noted on the left–lanes 4. The radioactivity of NHK co-transfected with ER ManI at 0 h chase was always weaker than that from cells co-transfected with the vector (pMH), lanes 4–6 were exposed twice as long as lanes 1–3. The open arrow indicates proteins that were co-immunoprecipitated with transfected ER ManI.

Inhibition of NHK Degradation by Lactacystin—The effect of the proteasome inhibitor lactacystin on NHK degradation was then examined. The accelerated degradation of NHK in cells transfected with ER ManI was repressed by lactacystin (Fig. 3A, arrow, compare lanes 1–3 with lanes 4–6), indicating that the enhanced NHK degradation induced by ER ManI overexpression occurs through the ERAD pathway. Again, it is notable that the transfected ER ManI is rapidly degraded (Fig. 3B, lanes 1–3, open arrow, see also Figs. 1B and 2B). General protein synthesis examined by the incorporation of [35S]methionine into the trichloroacetic acid-insoluble fraction was not inhibited by transfection of ER ManI. The degradation of ER ManI is independent of proteasomes (Fig. 3B, compare lanes 1–3 with lanes 4–6). The degradation of ER ManI was not inhibited by other protease inhibitors including serine or cysteine protease inhibitors, calpain inhibitor, nor aspartic protease inhibitor.2

Combined Effects of ER ManI and EDEM on NHK Degradation—Recently, we reported that mouse EDEM, which has sequence homology to α1,2-mannosidases but lacks enzyme activity with Man,GlCNac as substrate, accelerates ERAD of misfolded NHK when transfected into 293 cells (15). EDEM was shown to interact with NHK in co-immunoprecipitation experiments and was postulated to recognize misfolded glycoproteins to sort them for retrotranslocation. We first compared the effects of transfection with ER ManI and with EDEM on ERAD of NHK. ER ManI and EDEM expressed individually shortened the intracellular half-life of NHK (Fig. 4A, closed arrow, compare lanes 1–3 with lanes 4–6 or 7–9). Co-immunoprecipitation of either ER ManI or EDEM was detected with NHK, using antibody raised against A1AT (Fig. 4A, lanes 4–6, open arrow showing ER ManI; lanes 7–9, thin arrow indicating EDEM). In the immunoprecipitates using anti-HA antibody, protein bands of 170 and 150 kDa on SDS-PAGE were observed in ER ManI and EDEM transfected cells, respectively (Fig. 4B, lanes 4–6, open arrowhead for ER ManI; lanes 7–9, closed arrowhead for EDEM). Although transfected ER ManI is rapidly degraded, transfected

* N. Hosokawa, I. Wada, and K. Nagata, unpublished observation.
EDEM is stable during the 2-h chase period (Fig. 4B, compare lanes 4–6 with lanes 7–9).

Co-transfection of 293 cells with both ER ManI and EDEM further enhanced ERAD of NHK, compared with ER ManI alone, showing that there is a combined effect of these two ER resident proteins on NHK degradation (Fig. 5A, compare lanes 1–3 with lanes 4–6). Enhancement of degradation of NHK was also evident in cells co-transfected with both ER ManI and EDEM compared with EDEM alone (Fig. 5A, compare lanes 4–6 with lanes 7–9). Because of the rapid degradation of NHK observed following transfection of both ER ManI and EDEM in this experiment, cell lysates were examined at shorter chase periods. The levels of expression of ER ManI, EDEM, and NHK were reduced when both ER membrane proteins and substrate proteins were co-transfected. However, when the proteasome inhibitor lactacystin was added, similar amounts of labeled NHK were recovered from the immunoprecipitates of cell lysates (Fig. 5B, lanes 3, 4, 7, and 8), whereas only a very small amount of NHK was detected in cells overexpressing both ER ManI and EDEM after a 45-min chase in the absence of lactacystin (Fig. 5B, lane 6). These results confirm that the reduced amount of labeled NHK immunoprecipitated from cells co-transfected with ER ManI and EDEM was actually due to degradation by the proteasomes and not due to reduced synthesis of NHK.

We then investigated whether ER ManI and EDEM form a complex with each other. We prepared antisera against synthetic peptides from both ER ManI and EDEM. Each antiserum recognized its corresponding overexpressed protein by immunoprecipitation and Western blot analysis but hardly de-

FIG. 2. Kifunensine inhibits the degradation of NHK in ER ManI transfected cells. Kifunensine (5 μg/ml) was added to the culture medium 4 h prior to metabolic labeling and was present in the medium during the pulse-labeling and chase period. Both NHK and ER ManI were transfected in all the cells. Immunoprecipitates were prepared as in Fig. 1. Arrow notations are as described in Fig. 1. A and B, autoradiogram of the immunoprecipitates using antibody (Ab) against A1AT (A) and HA-tag (B), respectively. C, quantification of NHK within the cell. The radioactivity of NHK at 0 h chase was set arbitrarily to 1.0, and the means with standard deviations of three independent experiments were shown.

FIG. 3. Lactacystin inhibits the degradation of NHK in ER ManI transfected cells. Cells were transfected with ER ManI and NHK and treated with or without lactacystin (1 mM) for 4 h prior to metabolic labeling. In lactacystin + cells, drug was present during pulse-labeling and during chase. Autoradiogram of SDS-PAGE of immunoprecipitates are shown with anti-A1AT antibody (A) or with anti-HA-tag antibody (B). Arrows and an arrowhead indicate the same species as in Fig. 1.
 unprotected endogenous levels of the proteins. Accordingly, we co-
transfected HA-tagged ER ManI with untagged EDEM or un-
tagged ER ManI with HA-tagged EDEM. We performed
immunoprecipitation of labeled cell lysates, as well as immu-
noprecipitation of cell lysates followed by Western blotting
using either HA-tag or synthetic peptide antibodies. We did not
detect any interaction of overexpressed ER ManI with EDEM
(data not shown). The co-immunoprecipitated band observed in
cells transfected with ER ManI was consistently larger than
that observed in EDEM transfected cells (Fig. 4
B
, lanes 4
–
9,
open and closed arrowheads) indicating that the proteins
differ.

**Characterization of Oligosaccharides on NHK**—To deter-
mine the structure of the oligosaccharides present on NHK,
cells were labeled with [2-3H]mannose, and the oligosaccha-
rides released by Endo-H from immunoprecipitated NHK were
analyzed by HPLC (Fig. 6). In mock transfected cells the major
labeled oligosaccharides on NHK were Glc1Man9GlcNAc and
Man9GlcNAc following pulse-labeling, along with smaller
amounts of Man8GlcNAc (Fig. 6A). The identity of the oligosac-
charides was confirmed by exhaustive treatment with jack
bean α1,2-mannosidase followed by HPLC of the products.
Glc1Man9GlcNAc yielded about 47% labeled Glc1Man4GlcNAc
and mannose whereas Man9GlcNAc was entirely converted to
mannose (data not shown). With increasing times of chase,
trimming of NHK glycans to Man7GlcNAc, Man6GlcNAc (Fig.
6, B and C), and even to Man5GlcNAc after 4 h of chase (data
not shown) was observed. Therefore, the oligosaccharides on
misfolded NHK are trimmed beyond Man8GlcNAc by endoge-
nous α1,2-mannosidase activity.

Overexpression of ER ManI caused a large increase in
Man9GlcNAc, and an appearance of Glc1Man9GlcNAc
(Fig. 6, D, E, and F, and Fig. 7A). At the same time, there was
a large decrease in the proportion of Glc1Man9GlcNAc and Man9GlcNAc2 as well as an increase in Man5–7GlcNAc compared with mock transfected cells. An aliquot of the sample from NHK by Endo-H were fractionated by HPLC. Arrows indicate the position of the Glc1Man8GlcNAc internal standard. M5–M9 show 3H-labeled Man4GlcNAc, G1M8 corresponds to Glc1Man8GlcNAc (shown in Fig. 7), and G1M9, which co-eluted with 14C-labeled Glc1Man9GlcNAc (not shown), corresponds to Glc1Man9GlcNAc.

DISCUSSION

In the present study, we have shown that overexpression of human ER ManI in HEK 293 cells enhances ERAD of the terminally misfolded glycoprotein NHK. Accelerated degradation of NHK in cells overexpressing ER ManI was inhibited by lactacystin and by kifunensine, indicating that misfolded NHK was degraded through the ERAD pathway and that overexpression of ER ManI enhanced ERAD by accelerating trimming of its N-glycans. It has been postulated that the processing of N-linked oligosaccharides to Man8 by ER ManI acts as a signal for degradation of misfolded glycoproteins (1, 7, 13, 14).
However, all studies to date were based on the assumption that Man8B is the only oligosaccharide found on misfolded glycoproteins due to ER ManI activity and on observations of carbohydrate trimming from misfolded glycoproteins by shifts in their mobility upon SDS-PAGE. In this first study reporting HPLC analysis of the glycans on terminally misfolded NHK, the results clearly show that a mixture of oligosaccharides is found on NHK and that the accepted assumption that only Man8B is formed by ER ManI needs to be revised.

In mock transfected cells, Glc1Man9GlcNAc, Man9GlcNAc, and Man8GlcNAc were the major oligosaccharides found on NHK, but smaller amounts of Man7GlcNAc and Man6GlcNAc were also present. It is therefore possible that oligosaccharides other than Man8B, or in addition to Man8B, may act as recognition markers for ERAD. The relatively small amounts of Man7GlcNAc and Man6GlcNAc found on NHK might be the result of selective ERAD of specific NHK glycoforms bearing these oligosaccharides. The presence of Glc1Man9GlcNAc on NHK is consistent with previous studies demonstrating NHK association with calnexin (26). The results also show that the additional trimming beyond Man8GlcNAc is not due to ER α-mannosidase II because it was sensitive to kifunensine (Fig. 8).

Overexpression of ER ManI, which increased ERAD of NHK, also greatly stimulated trimming of mannose residues to Man7GlcNAc, Man6GlcNAc, and Man5GlcNAc, as well as the formation of Glc1Man8GlcNAc. With the exception of the latter, the same oligosaccharides were formed in mock transfected cells, showing that the stimulation of NHK degradation caused by overexpression of ER ManI is not likely caused by the formation of atypical glycans. The increased trimming of N-glycans beyond Man6GlcNAc observed in cells overexpressing ER ManI is consistent with previous experiments with recombinant ER ManI in vitro indicating that ER ManI is less specific than previously believed (25).

The pattern of oligosaccharides found in EDEM and mock transfected cells is very similar, but there is always a shoulder ahead of the Man6GlcNAc peak that corresponds to a small
amount of Glc$_1$Man$_9$GlcNAc, suggesting that EDEM somehow facilitates either trimming of Glc$_1$Man$_9$GlcNAc to Glc$_1$Man$_8$GlcNAc or glucosylation of Man$_8$GlcNAc by UDP-glucose: glycoprotein glucosyltransferase. We demonstrated combined effects of ER ManI and of EDEM on ERAD. We previously reported that EDEM interacts with and accelerates the degradation of misfolded NHK, suggesting that EDEM may be a putative lectin, which recognizes misfolded glycoproteins for ERAD (15). NHK degradation is faster in cells co-transfected with both ER ManI and EDEM than in cells transfected with ER ManI or EDEM alone (Fig. 5A). We also obtained evidence indicating that ER ManI and EDEM are part of different complexes because different proteins were co-immunoprecipitated with each of them, and no evidence that ER ManI and EDEM form a complex with each other was obtained. We propose a model whereby misfolded glycoproteins are recognized independently by ER ManI and by EDEM. The identities of the proteins co-immunoprecipitated with ER ManI and EDEM remain to be determined, which may aid in the elucidation of productive glycoprotein folding and in understanding the machinery leading to retrotranslocation and subsequent degradation by proteasomes. Notably, ER ManI transfected into 293 cells was degraded very rapidly through a mechanism independent of cytoplasmic proteasomes (Fig. 3B, lanes 1–6). Despite its rapid turnover, we could detect the expression of transfected ER ManI by Western blot analysis using antibody against HA-tag or human ER ManI peptide. The level of expression of ER ManI in transfected cells is higher than that of endogenous ER ManI because we could not detect the latter by Western blot analysis using anti-peptide antibody. Although the molecular mechanism and the reason for the rapid degradation of ER ManI will require further investigation, the results suggest that the level of ER ManI expression is stringently controlled.

Acknowledgments—We thank Bjorn Stork for assistance in construct preparation and Dr. Pedro Romero for comments on the manuscript.

REFERENCES
1. Ellgaard, L., Molinari, M., and Helenius, A. (1999) Science 286, 1882–1888
2. Herscovics, A. (1999) Biochem. Biophys. Acta 1473, 96–107
3. Parodi, A. J. (2000) Annu. Rev. Biochem. 69, 69–93
4. Helenius, A., and Aebi, M. (2001) Science 291, 2364–2369
5. Bonifacino, J. S., and Weissman, A. M. (1998) Annu. Rev. Cell Dev. Biol. 14, 19–57
6. Pemper, R. K., and Wolf, D. H. (1999) Trends Biochem. Sci. 24, 266–270
7. Cabral, C. M., Liu, Y., and Sifers, R. (2001) Trends Biochem. Sci. 26, 619–624
8. Gonzalez, D. S., Karaveg, K., Vandersall-Nairn, A. S., Lal, A., and Moremen, K. W. (1999) J. Biol. Chem. 274, 21375–21386
9. Tremblay, L. O., and Herscovics, A. (1999) Glycobiochemistry 9, 1073–1078
10. Knop, M., Hauser, N., and Wolf, D. H. (1996) Yeast 12, 1229–1238
11. Jakob, C. A., Burda, P., Roth J., and Aebi, M. (1996) J. Cell Biol. 122, 1223–1233
12. Liu, Y., Choudhury, P., Cabral, C. M., and Sifers, R. N. (1999) J. Biol. Chem. 274, 5861–5867
13. Tokunaga, P., Brotrom, C., Koid, T., and Arvan, P. (2000) J. Biol. Chem. 275, 40757–40764
14. Fagiolii, C., and Sitia, R. (2001) J. Biol. Chem. 276, 12885–12892
15. Hosokawa, N., Wada, I., Hasegawa, K., Yorihuzi, T., Tremblay, L. O., Herscovics, A., and Nagata, K. (2001) EMBO Rep. 2, 415–422
16. Jakob, C. A., Bodmer, D., Spirig, U., Bättig, P., March, D., Dignard, D., Berge, J. M., Thomas, D. Y., and Aebi, M. (2001) EMBO Rep. 2, 423–430
17. Nakatsukasa, K., Nishikawa, S. I., Hosokawa, N., Nagata, K., and Endo, T. (2001) J. Biol. Chem. 276, 8633–8638
18. Sifers, R. N., Brahaers-Macatee, S., Kidd, V. J., Krueger, H., and Wu, S. L. C. (1988) J. Biol. Chem. 263, 7330–7335
19. Satoh, M., Hirayoshi, K, Yokota, S-i., Hosokawa, N., and Nagata, K. (1996) J. Cell Biol. 133, 469–483
20. Moore, S. E. H., and Spiro, R. G. (1984) J. Biol. Chem. 269, 12715–12721
21. Romero, P. A., Saunier, B., and Herscovics, A. (1985) Biochem. J. 226, 733–740
22. Lipari, P., and Herscovics, A. (1999) Biochemistry 38, 1111–1118
23. Cox, D. W. (1985) In Scrivener, C. B., Beaudet, A. L., Sly, W. S., and Valle, D. (eds) The Metabolic Basis of Inherited Disease, pp. 4125–4158, McGraw-Hill, New York
24. Liu, Y., Choudhury, P., Cabral, C. M., and Sifers, R. N. (1997) J. Biol. Chem. 272, 7846–7851
25. Herscovics, A., Romero, P.A., and Tremblay, L. O. (2002) Glycobiochemistry 12, 14G–15G
26. Le, A., Stein, J. L., Ferrell, G. A., Shaker, J. C., and Sifers, R. N. (1994) J. Biol. Chem. 269, 7514–7519
Enhancement of Endoplasmic Reticulum (ER) Degradation of Misfolded Null Hong Kong α1-Antitrypsin by Human ER Mannosidase I
Nobuko Hosokawa, Linda O. Tremblay, Zhipeng You, Annette Herscovics, Ikuo Wada and Kazuhiro Nagata

J. Biol. Chem. 2003, 278:26287-26294.
doi: 10.1074/jbc.M303395200 originally published online May 6, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303395200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 25 references, 16 of which can be accessed free at http://www.jbc.org/content/278/28/26287.full.html#ref-list-1