Proteins expressed in the endoplasmic reticulum (ER) are subjected to a tight quality control. Persistent association with ER-resident molecular chaperones prevents exit of misfolded or incompletely assembled polypeptides from the ER and forward transport along the secretory line. ER-associated degradation (ERAD) is in place to avoid ER constipation. Folding-incompetent products have to be identified to interrupt futile folding attempts and then targeted for unfolding and dislocation into the cytosol for proteasome-mediated destruction. These processes are better understood for N-glycosylated proteins that represent the majority of polypeptides expressed in the ER. EDEM, a mannosidase-like chaperone, regulates the extraction of misfolded glycoproteins from the calnexin cycle. Here we identify and characterize EDEM2, a novel, stress-regulated mannosidase-like protein that operates in the ER lumen. We show that transcriptional up-regulation of EDEM2 depends on the ER stress-activated transcription factor Xbp1, that EDEM2 up-regulation selectively accelerates ERAD of terminally misfolded glycoproteins by facilitating their extraction from the calnexin cycle, and that the previously characterized homolog EDEM is also a soluble protein of the ER lumen in HEK293 cells.

Many of the proteins that fold in the ER\(^1\) are covalently modified by co-translational addition of pre-assembled glycans to an Asn-X-Ser/Thr consensus sequence. Only recently has the importance of these carbohydrate tags been fully appreciated. N-Linked glycans can increase the solubility of unstructured intermediates of the protein folding process thereby inhibiting the formation of toxic aggregates. Each of the sugar units composing the protein-bound glycan plays a role in the transfer of the branched glycan from the lipid donor in the ER membrane to nascent polypeptides, in mediating binding/release from ER resident lectins, and/or in determining the fate of the associated polypeptide chain (1). Glucose trimming by glucosidase I and glucosidase II and glucose re-addition by UDP-glucose:glycoprotein glucosyltransferase (GT) allows nascent and newly synthesized proteins access to and cycling into a system of chaperones composed by the homologous lectins calnexin (Cnx) and calreticulin (Crt) and the associated oxidoreductase ERp57 (2–5). Native proteins are transported along the secretory line to their destination, but a certain fraction of newly synthesized glycoproteins will never make it into a native, export-competent structure. For proteins such as the cystic fibrosis channel the percentage of folding-incompetent chains may be as high as 80\% (6). Amino acid mutations or deletions may further increase the amount of protein to be destined for ERAD.

A tightly controlled extraction of folding-incompetent polypeptides from the Cnx cycle and their selection for ERAD is crucial to prevent ER constipation and to maintain productive folding assistance by the ER chaperone network (7). Extraction is regulated by two members of the family 47 glycosyl hydrolases, ER \(\alpha\)-mannosidase I and EDEM (ER degradation-enhancing \(\alpha\)-mannosidase like protein; Ref. 8). ER \(\alpha\)-mannosidase I cleaves one (9) or more (10) mannose residues from protein-bound N-glycans. This makes glucosidase-mediated protein de-glucosylation (11) and GT-mediated protein re-glucosylation (12) less efficient and slows down the on/off cycles of substrate binding to/release from Cnx. Moreover, N-glycans with a reduced number of mannoses, if coupled to misfolded protein determinants, may determine deviation of the polypeptide chain into the ERAD machinery. Mannose trimming may therefore eventually lead to active extraction of folding incompetent proteins from the folding attempts phase in the Cnx cycle (13–20). Consistent with this, selective inhibition of ER \(\alpha\)-mannosidase I with the alkaid kifunensine (Refs. 21 and 22) prevents hydrolysis of the Man9 structure (10), retards release of terminally misfolded glycoproteins from the Cnx cycle (23), and delays their degradation (reviewed in Ref. 14).

EDEM is a stress-regulated protein (8). Defective adaptation of its expression level to variations in ER cargo load or to the accumulation of misfolded glycoproteins is obtained in cells exposed to small interfering RNA targeting EDEM transcripts (19), in cells depleted of the proximal ER stress sensor IRE1\(\alpha\) (24), or in cells depleted of the IRE1\(\alpha\)-activated transcription factor Xbp1 (7) and delays ERAD of glycoproteins. Under these conditions, by-products of protein biosynthesis progressively accumulate in the ER lumen eventually compromising secretory capacity (7).

A data base search using mouse EDEM revealed two novel homologues in both the mouse and human genomes which were members of the glycosidase 47 family. One of these proteins, named here EDEM2, was selected for further study because of the similarity to EDEM and the fact that the corresponding gene was up-regulated in response to ER stress in an Xbp1-de-
pendent manner. The intracellular localization and consequences of overexpression on the ERAD of glycoprotein were analyzed for EDEM and for EDEM2. EDEM, a type 2 membrane protein in Cos cells (8), and EDEM2 are soluble glycoproteins of the ER lumen in human embryonic kidney cells (HEK). EDEM2 showed a similar capacity to EDEM to accelerate the extraction of folding-incompetent glycoproteins from the Cnx cycle and their disposal from the ER, without affecting the rate of degradation of non-glycosylated polypeptides or the maturation of model secretory proteins.

**EXPERIMENTAL PROCEDURES**

**Mammalian Expression Constructs and Antibodies—Plasmids for expression of EDEM and EDEM2 with a C-terminal EFRH tag were generated by PCR from IMAGE clones 2654113 and 2811074, respectively. The PCR products were cloned into the mammalian expression vector pRK7. The polycistronic anti-Cnx was a kind gift of A. Helenius (Zurich). The monoclonal anti-EFRH used for immunoprecipitation and immunofluorescence of EFRH-tagged EDEM and EDEM2 was a kind gift of P. Paganetti (Basel, Switzerland). Anti-HA used for immunoprecipitation and immunofluorescence of HA-tagged EDEM was from Sigma. P. Paganetti’s anti-EDEM antibody was a kind gift of Steve High (Manchester, UK).

**Transfections—**Cells were transiently transfected with Lipofectamine 2000 (Invitrogen) according to the instructions of the manufacturer. Briefly, HEK293 cells were seeded at 80–90% confluence in polylysine-coated 6-cm Petri dishes. Eight hours later they were supplemented with 4 μg of expression plasmid in 15 μl of Lipofectamine 2000 diluted in 800 μl of Opti-MEM (Invitrogen/dish). For co-transfections (EDEM or EDEM2 and BACE476) the DNA for EDEM/EDEM2 was added in 2-fold excess. Expression plasmids containing a selection gene for Geneticin resistance were linearized for stable transfections. Selection media (2% Geneticin) was added to cells 48 h after transfection, and individual geneticin-resistant clones were isolated 12 days after transfection. Expression of EDEM or EDEM2 of the individual clones was assessed by immunoprecipitation and immunoblot with HA- or EFRH tag specific antibodies.

**Pulse Chase and Immunoprecipitations—**Cells were starved for 30 min in Met/Cys-free medium, pulsed for 10 min with 150 μCi of [35S]Met/Cys in 1 ml of starvation medium/dish, and chased for the times indicated in the figures with Dulbecco’s modified Eagle’s medium supplemented with 5 mM cold Met/Cys. Postnuclear supernatants were prepared by solubilization of cells in 800 μl/dish ice-cold 2% CHAPS in Heps buffer saline, pH 6.8, containing 20 mM N-ethylmaleimide, protease inhibitors (HBS). Cell extracts were prepared by 10 min centrifugation at 10,000 × g and analyzed by reducing SDS-PAGE. Immunoprecipitations were performed by adding protein A beads (Sigma; 1:10 v/w swollen in HBS) and the selected antibody to the cell extracts. Incubations were 1–4 h in a cold room. The immunoprecipitates were extensively washed, three times, with HBS, 0.5% CHAPS and resuspended in sample buffer for SDS-PAGE. Relevant bands were quantitated by ImageQuant software (Amersham Biosciences). Gels were also exposed to BioMax (Eastman Kodak) films and scanned with an Agfa scanner. For EndoH (Roche Applied Science) treatment, immunoprecipitated BACE476 was denatured and then incubated for 1 h at 37 °C with 5 milliunits of Endo H.

**Subcellular Fractionation and Preparation of Membranes Versus Luminal Content—**Metabolically labeled cells were extensively washed in a 10-cm dish with isotonic buffer. They were gently detached with a rubber policeman and resuspended in 800 μl of homogenization buffer (10 mM triethanolamine, 10 mM acetic acid, 250 mM sucrose, 20 mM N-ethylmaleimide, 1 mM EDTA, and a mixture of protease inhibitors, pH 7.4). Cells were broken with 10–15 passages through a 22-gauge 1 and ¼ needle. Postnuclear supernatants were subjected to a first ultracentrifugation (100,000 × g in TLA 120.2) to separate endomembranes from the cytosol. The endomembrane-containing pellet was washed twice and left for 25 min on ice in 500 μl of 100 mM Na2CO3, for carbonate extraction of the luminal content. After an additional ultracentrifugation step (100,000 × g in TLA 120.2), the luminal fraction was harvested and supplemented with antibodies to Cnx, Crt, or to EDEM to determine their presence. The endomembrane fraction was subjected to an additional washing step with 100 mM Na2CO3, to minimize luminal contaminations and then resuspended in 800 μl of lysis buffer (2% CHAPS). The lysate was subjected to ultracentrifugation to eliminate insoluble material and the supernatant was analyzed by immunoprecipitation for chaperone content.

**Semiquantitative Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)—**1 × 106 wt mouse embryonic fibroblasts (MEF) or XBP-1 cells were plated in 6-well dishes without (mock) or with tunicamycin (Tun, 2.5 μg/ml). After 12 h cells were lysed in TRIzol reagent (Invitrogen) and RNA isolated according to the instructions of the manufacturer. Two micrograms of RNA were used for cDNA production by SuperScriptII reverse transcriptase (Invitrogen) primed by oligo(dT) (Invitrogen). Semiquantitative PCR was performed using TaqDNA polymerase (Invitrogen) with transcript-specific primers using three different cycle numbers for each gene, all within the linear phase of template amplification. The primers were as follows: β-actin, CTTTC-TGGTATGGAAATCTT (forward) and GGAGCAATGTCTTGGTATCTT (reverse); BIP, GAGTTCTCAATGGGGAAGAGGA (forward) and CCGT-CAGTCAATGATGAC (reverse); EDEM, GGGAGGCCGTGAGATA (forward) and CCAAACTGCTCCGATAC (reverse); EDEM3, ATCAGAATTTGGGTTGCTT (forward) and TCGACATGTTTTGGTGGATA (reverse).

**Signal Peptide Predictions—**SignalP 3.0 (25) and PSORT II (26) were used to predict signal peptide versus signal anchor sequences in mouse ER α-mannosidase, EDEM, and EDEM2. Both programs predicted that EDEM and EDEM2 contained probable cleavable signal peptides, while α-mannosidase I contained a probable signal anchor. For the hidden Markov model used by SignalP, EDEM had a signal peptide probability of 0.996, EDEM2 a signal peptide probability of 0.999, and α-mannosidase I a signal anchor probability of 0.951.

**RESULTS**

**Identification of a Novel EDEM-like Protein—**A data base search for novel components of ERAD using either mouse EDEM (8) or the yeast ER α-mannosidase-like protein Htp1p (27) identified two previously uncharacterized open reading frames in each of the mouse and human genomes. All four proteins were members of the family 47 glycosyl hydrolases, which includes both EDEM and ER α-mannosidase I. The first gene, here named EDEM, encoded a 577 amino acid protein in mouse (Swiss-Prot accession number Q91V3C, NCBI nucleotide 95300900G24Rik), which was 94% identical to a 578 amino acid protein in human (Swiss-Prot accession number Q8QV94; NCBI nucleotide C20rfr31). The second pair of genes, here tentatively named EDEM3, encoded a 931-aa amino acid protein in mouse (NCBI nucleotide 2310050N11Rik), which from Met25 was 95% identical to a 889 amino acid protein in human (Swiss-Prot accession number Q5BZQ6, NCBI nucleotide C1orf22). The EDEM3 proteins not only contain a family 47 glycosyl hydrolase domain but also a protease-associated domain that could serve as an interaction site between EDEM3 and incorrectly folded proteins. EDEM3 is significantly longer than either EDEM, EDEM2, or Htp1p. No studies have yet been undertaken on EDEM3.

Unigene (28) cDNA sources for mouse EDEM2 include bone, brain, colon, eye, heart, kidney, liver, lung, lymph node, mammary gland, ovary, pancreas, placenta, pituitary gland, skin, spleen, stomach, testis, thymus, and uterus.

An alignment of EDEM2 with EDEM and ER α-mannosidase I reveals that the core family 47 glycosyl hydrolase domain is relatively well conserved but that there is little or no conservation beyond this domain. The identities in the family 47 glycosyl hydrolase domain, however, are 44% for mouse EDEM versus mouse α-mannosidase 2, 35% for mouse EDEM versus human ER α-mannosidase I, and 32% for mouse EDEM2 versus human ER α-mannosidase I. The mannosidase catalytic residues are conserved in EDEM and EDEM2, with the exception of a pair of cysteines that are conserved in most catalytically active mannosidases. The lack of these cysteines has been reported to be responsible for the lack of enzymatic activity of EDEM (8). Mouse EDEM2 has four potential N-glycosylation sites, all of which are conserved in the human protein. EDEM2 is highly conserved between mouse, human, chicken, and xenopus except for a sequence corresponding to amino acids 508–553 of mouse EDEM2. Analysis of the EDEM2 sequence revealed that...
it has a predicted cleavable signal sequence with a probability exceeding 99%. Similar analysis of EDEM revealed a predicted cleavable signal sequence with a probability exceeding 99%, while ER-α-mannosidase I is identified by the same algorithms as being a type 2 membrane protein.

**EDEM and EDEM2 Are N-Glycosylated Proteins of the ER Lumen in HEK293 Cells**—HEK293 cells were transfected for stable or transient expression of tagged versions of EDEM or EDEM2. The tags used in our studies were a conventional nonapeptide HA tag (YPYDVPDYA) and a shorter tetrapeptide tag (EFRH) for which monoclonal antibodies are available in our laboratory. The tags were placed at the C termini of EDEM and EDEM2 as it had been previously shown that a C-terminal tag did not affect intracellular localization and activity of EDEM (8).

Expression, N-glycosylation, and intracellular localization of EDEM and EDEM2 were assessed after metabolic labeling of cells. Transfected cells expressing the tagged versions of EDEM or EDEM2 were incubated for 10 min with [35S]methionine and cysteine and chased in a medium containing excess of unlabeled methionine and cysteine before lysis with CHAPS. Postnuclear supernatants were immunoprecipitated with the specific anti-tag antibody and proteins were separated by reducing SDS-PAGE gels. EDEM-EFRH (shown in Fig. 1A) and EDEM-HA (data not shown) had an apparent molecular mass of 78 kDa; EDEM2-EFRH was about 70 kDa (Fig. 1A). Endo H treatment of the immunopurified recombinant proteins revealed that EDEM and EDEM2 were extensively glycosylated in HEK293 cells, consistent with the presence of five and four consensus sequences for N-glycosylation respectively. The mobility shift was the same just after synthesis when the proteins are in the ER and all N-glycans are Endo H-sensitive and 2 h after synthesis, as a clear indication that all glycans had maintained Endo H sensitivity (Fig. 1B) and that the proteins were not transported to the Golgi compartment (29). The localization of EDEM and EDEM2 in the ER was also confirmed by immunofluorescence (data not shown).

To determine whether EDEM and EDEM2 expressed in HEK293 cells are luminal or transmembrane proteins mock-transfected cells and cells with stable expression of EDEM or EDEM2 were metabolically labeled, extensively washed, and broken with 10–13 passages through a 22-gauge 1 and ¼ needle. Postnuclear supernatants were subjected to a first ultracentrifugation to separate endomembranes from the cytosol. The endomembrane-containing pellet was washed twice before carbonate extraction of the luminal content. The luminal fraction was supplemented with antibodies to Cnx, Crt, or to “tagged” EDEM to determine their presence. The endomembrane fraction was subjected to an additional washing step to reduce luminal contaminations and then detergent-solubilized before immunoprecipitation with antibody to Cnx, Crt, or to recombinant and endogenous EDEM. Samples were analyzed by SDS-PAGE (Fig. 1, C–F).

In mock-transfected cells, 90% of the labeled Crt was isolated from the luminal (Fig. 1C, lane 1) and 10% from the endomembrane fraction (lane 4) indicating incomplete rupture of intracellular compartments or tight association of Crt with a membrane-bound ER component. Cnx was solely extracted from the membrane fraction (lane 5), as expected for this type I ER membrane protein.

The tagged versions of EDEM and EDEM2 were isolated from the luminal fraction of HEK293 cells (Fig. 1D, lane 9, and E, lane 15, respectively). For EDEM the result was unexpected because this protein is membrane-bound in COS cells (8). Our results, however, confirmed predictions based on the protein sequence (see above). No change was observed in the localization of Cnx or Crt in the EDEM or EDEM2 overexpressing cells (Fig. 1, D, lanes 7 and 8 and 10 and 11 and E, lanes 13 and 14 and 16 and 17). A small amount of EDEM (asterisk in Fig. 1D, lane 12) and EDEM2 (+ in Fig. 1E, lane 18) was also found in the membrane fraction. These immunoreactive proteins were not present in the membrane fraction of mock-transfected cells (lane 6) and had gel mobility slightly slower than the EDEM found in the ER lumen. Thus, they most likely represent the fraction of recombinant EDEM and EDEM2 remaining anchored to the membrane through uncleaved signal peptide. The prevalent luminal localization of endogenous EDEM in the ER lumen of HEK293 cells was also confirmed (arrow in Fig. 1F).

**EDEM and EDEM2 Are Stress-regulated Proteins of the ER Lumen**—An increase in cargo load or accumulation of misfolded polypeptides in the ER lumen trigger an unfolded protein response (UPR) with transcriptional up-regulation of a plethora of genes involved in protein and lipid biosynthesis, protein maturation, protein degradation, and protein transport along the secretory route (30). Depletion of the proximal ER stress sensor IRE1α or of the transcription factor Xbp1 only mildly affects...
induction of BiP/Grp78 and of other ER resident chaperones but abolishes EDEM up-regulation upon ER stress (24, 31).

We first determined whether the novel member of the glycosyl hydrolase 47 family, EDEM2, is a stress-regulated gene and whether Xbp1 plays a role in its regulation. To this end, MEF and MEF derived from a Xbp1 knock-out mouse (Xbp1−/−) were mock-treated or exposed for 12 h to Tun, an inhibitor of protein N-glycosylation that triggers UPR. At 2.5 μg/ml, Tun was inhibited by 50% N-glycosylation of two control viral glycoproteins (data not shown). Although up-regulation of ER stress-responding chaperones was not maximal (data not shown), this concentration of Tun was chosen for our experiments as it did not affect cell viability. To monitor variations in the expression of ER chaperones, RNA was isolated from mock- and Tun-treated wt and Xbp1−/− MEF and RT-PCR was performed followed by semiquantitative PCR with chaperone-specific primers.

ER stress induction of BiP/Grp78 is only weakly affected in cells with defective Ire1α/Xbp1 response (24). Accordingly, exposure of wt MEF (Fig. 1G, compare lanes 1 and 2) and of Xbp1−/− MEF (Fig. 1G, lanes 3 and 4) to Tun resulted in comparable induction of BiP. On the other hand, EDEM induction requires a fully functional Ire1α/Xbp1 response (24, 31). Not surprisingly, Tun triggered transcriptional up-regulation of EDEM in wt MEF (Fig. 1G, lanes 9 and 10), but it failed to do so in cells depleted of Xbp1 (Fig. 1G, lanes 11 and 12). EDEM2 is also an ER stress-responding protein as it was clearly up-regulated in wt MEF exposed to Tun (Fig. 1G, lanes 13 and 14). As for EDEM, Xbp1 depletion abolished EDEM2 induction (Fig. 1G, lanes 15 and 16). EDEM3 transcription was on the other hand not enhanced upon ER stress (Fig. 1G, lanes 17–20).

Thus, EDEM (24, 31), EDEM2 (this work), and the ER α-mannosidase I (32) are three members of the glycosidase 47 family that all rely on the transcription factor Xbp1 for ER stress-induced up-regulation. Since EDEM and the ER α-mannosidase I are involved in ERAD of glycoproteins, our results prompted us to analyze the consequences of EDEM2 up-regulation in degradation from the ER of a model glycoprotein with defective folding.

Overexpression of EDEM2 Accelerates Glycoprotein Degradation from the ER—An increase in the intracellular level of EDEM artificially obtained by cell transfection with EDEM expression plasmids or more physiologically during an UPR has been shown to improve the capacity to clear aberrant glycoproteptides from the ER lumen in mammalian and yeast cells (7, 8, 18–20, 24, 27, 33, 34).

We determined whether up-regulation of EDEM2 affected degradation of a model ERAD substrate, namely BACE476, a tetraglycosylated type 1 membrane protein, has been determined in HEK293 cells and in the same cells with higher expression of EDEM or EDEM2. EDEM and EDEM2 overexpression reduces the length of the lag phase preceding onset of BACE476 degradation and drastically shortens the protein half-life. B, degradation of the same protein devoid of N-glycans expressed in the same cells is not affected by EDEM and EDEM2 overexpression. C, closer analysis of the lag phase. In the first hour after protein synthesis the amount of BACE476 does not change in HEK293 cells (Mock). The non-glycosylated protein is rapidly degraded. Upon EDEM or EDEM2 overexpression a significant decrease of BACE476 content occurs between the 30- and the 60-min chase. D, detergent extracts were immunoprecipitated with Cnx-specific antibodies to isolate Cnx-substrate complexes. EDEM and EDEM2 overexpression accelerate BACE476 release from Cnx.

We also determined whether overexpression of EDEM2 affected degradation of a model ERAD substrate, namely BACE476, a tetraglycosylated splice variant of human β-secretase with defective folding in mammalian cells and of BACE476NOG, the same protein without N-glycans. The kinetics of BACE476 ERAD was measured in mock-transfected HEK293 cells and in cells with increased expression of EDEM or EDEM2 (Fig. 2A). Cells were metabolically labeled as described above and chased for the times shown in Fig. 2A. Labeled BACE476 and BACE476NOG were immunoprecipitated with a specific antibody from detergent extracts, separated in SDS-PAGE, and quantified.

The amount of labeled BACE476 remained virtually constant for about 60 min in HEK293 cells (lag, Fig. 2, A and C, Mock) before the onset of degradation. In contrast, labeled BACE476NOG was rapidly degraded in these cells (Figs. 2, B and C, Mock). Overexpression of EDEM and of EDEM2 significantly reduced the length of the lag phase preceding disposal of BACE476 (Fig. 2A). After a 60-min chase less than 5% BACE476 had been degraded in wt cells versus 32 and 27% in cells overexpressing EDEM or EDEM2, respectively (average of three independent experiments, Fig. 2C). Earlier ERAD onset in cells expressing high levels of EDEM and EDEM2 correlated with premature release of BACE476 from Cnx (Fig. 2D), further confirming the protection from ERAD offered to folding-defective glycoproteptides by persistent retention in the Cnx cycle.

As shown previously for EDEM (8, 18, 19, 27, 34), overexpression of EDEM2 did not affect the degradation of a non-glycosylated protein (BACE476NOG in Figs. 2, B and C). The premature onset of ERAD in cells with a higher expression of EDEM and EDEM2 eventually resulted in a shortening of the half-life of BACE476 from about 240 to 120 min (Fig. 2A).

DISCUSSION

Proteins expressed in the ER are delivered to their final intra- or extracellular destination only after having acquired native structure. ER quality control retains aberrant polypeptides in the ER lumen, and ERAD insures their disposal thus preserving efficiency of the protein factory. The protein degradation activity must be finely tuned because under-activity may lead to accumulation and eventually to deposition of ab-

![Image](https://example.com/image.png)
errant polypeptides in intra-lumenal (7) or cytosolic (35) aggregates that may compromise secretory capacity or viability, respectively. Hyperactivity of ERAD may on the contrary cause extraction of folding intermediates from the folding attempts phase and their premature disposal (20, 36, 37). These data hint at a competition between folding and degradation machineries for folding-competent and folding-incompetent polypeptides expressed in the ER, similarly to what happens for polypeptides expressed in the cytosol (38). Any increase in ER cargo load due to increased protein synthesis or to abnormal persistence of polypeptides in the ER lumen demands prompt regulation of ERAD capacity as part of the cell adaptation program, as an indication that the activity of the ERAD machinery is maintained low under normal conditions.

Here we have characterized EDEM2, a novel member of the family 47 glycosyl hydrolases, which includes both EDEM and ER αmannosidase I, two ER-resident proteins involved in the regulation of degradation of terminally misfolded glycoproteins from the ER. EDEM2 localizes in the lumen of the ER. We report here that endogenous and recombinantly expressed EDEM shown here and in previous work (7, 8, 10, 19, 20) to report here that endogenous and recombinantly expressed from the ER. EDEM2 localizes in the lumen of the ER. We have characterized EDEM2, a novel member of the family 47 glycosyl hydrolases, which includes both EDEM and ER α-mannosidase I, two ER-resident proteins involved in the regulation of degradation of terminally misfolded glycoproteins from the ER. EDEM2 localizes in the lumen of the ER. We report here that endogenous and recombinantly expressed EDEM shown here and in previous work (7, 8, 10, 19, 20) to accelerate ERAD of glycoproteins in HEK293 cells is also a soluble ER protein. This is consistent with algorithm-based analysis of the EDEM primary sequence predicting efficient cleavage of the signal peptide, but it is in contrast with previous work showing that EDEM is a type 2 membrane protein in Cos cells (8). Evidently, the efficiency of the signal peptide cleavage of EDEM varies in different cell lines. Intriguingly, EDEM overexpression does not accelerate ERAD in all cell lines tested so far (20). It is possible that EDEM release from the ER membrane plays a regulatory role in EDEM activation.

We report here that EDEM2 is a stress-responsive luminal protein of the ER and constitutes with EDEM (24, 31) and the ER α-mannosidase I (32), a triad of genes involved in degradation of misfolded glycoproteins from the ER lumen whose induction depends on the stress-activated transcription factor Xbp1. The IRE1/Xbp1 UPR pathway, until recently considered as “accessory” to the work of other players during mammalian UPR (39), has been found to play an essential role in generation and survival of secretory cells like hepatocytes and antibody-secreting plasma cells (40–43). Cumulating evidence showing substantial dependence of ERAD activity on a functional IRE1/ Xbp1 UPR pathway (Refs. 7 and 24 and this work) and finding that ERAD activity and secretory capacity are intimately related (7) underscore the relevance of the Xbp1-regulated modulation of components of the ERAD machinery for generation, survival, and unperturbed activity of secretory cells.

Acknowledgments—We thank L. Bolliger, C. Jakob, and T. Solda for helpful discussions; we are greatly indebted to A. Helenius and S. High for antibodies, to L. Glimcher for Xbp1−/−MEF, to K. Nagata and N. Hosokawa for the EDEM-HA, and to P. Pagani for the BACE476 expression vectors, and to G. Noseda. We thank Riccardo Vago for the EDEM2 RT-PCR.