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Folding and self-assembly do not prevent ER retention and proteasomal degradation of asialoglycoprotein receptor H2a

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Abstract The human asialoglycoprotein receptor H2a precursor, a type II membrane protein, is cleaved to a soluble form that is secreted. Uncleaved precursor molecules are completely retained in the endoplasmic reticulum (ER) and degraded by the proteasome. To find out the causes of its fate we studied folding of H2a precursor, which was very similar to that of its alternatively spliced variant H2b which can exit to the Golgi. Proteasomal inhibition led to accumulation of folded rather than unfolded molecules. Accumulation of ER-retained H2a did not cause an unfolded protein response. Although the receptor is a heterooligomer of the H1 and H2 subunits, single expression led to some self-assembly. Whereas these homooligomers accumulated for H2b they were degraded for H2a. Translocation of H2a into the ER occurred efficiently. Therefore, the retention and proteasomal degradation of uncleaved membrane-bound H2a precursor from the ER do not involve aberrant translocation or misfolding and are not prevented by self-assembly.

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Key words: Endoplasmic reticulum degradation; Proteasome; Asialoglycoprotein receptor; T cell receptor; Assembly; Folding

1. Introduction

The endoplasmic reticulum (ER) quality control apparatus has as its main function the retention and degradation through the proteasomal pathway of misfolded proteins and of unassembled subunits of oligomers [1–3]. Precursors that fail to be cleaved are also retained and degraded as is the case with proteins that contain an uncleaved glycosylphosphatidylinositol signal [4]. This is also the case with membrane-bound human asialoglycoprotein receptor (ASGPR) H2a precursor, which is retained and degraded if it is not successfully cleaved to give a soluble secreted form [5]. The retention of H2a precursor was shown to involve a pentapeptide, EGHRG, in its ectodomain next to the membrane-spanning region. Introduction of this pentapeptide into the H1 subunit of the receptor (normally expressed at the cell surface) caused its complete ER retention but the protein remained stable [6]. It is usually assumed that proper folding and assembly preclude retention and degradation. However, retained proteins with an uncleaved glycosylphosphatidylinositol signal fold properly [7] and so does ASGPR H1 after introduction of the H2a pentapeptide [6]. Deficient translocation was also shown to cause ER retention and degradation as is the case with ApoB [8,9]. Here we show that H2a precursor folds and also self-assembles similarly to its splicing variant H2b (which does not contain the EGHRG signal and is not ER-retained). However, the folding and self-assembly of H2a do not preclude its ER retention and degradation through the proteasomal pathway. We also show that the juxtamembrane pentapeptide does not alter the efficiency of translocation of H2a.

2. Materials and methods

2.1. Materials

Rainbow ^{14}C-labeled methylated protein standards were obtain from Amersham (Buckinghamshire, UK). Pro-mix cell labeling mix ([^{35}S]methionine plus [^{35}S]cysteine) was from Amersham; >1000 Ci/mmol. Protein A-Sepharose was from Repligen (Cambridge, MA, USA). *N*-Acetyl-leucyl-leucyl-norleucinal (ALLN) and *N*-glycosidase F were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN, USA). *N*-Carbobenzoxyl-leucinyl-leucinyl-leucinal (MG-132) was from Calbiochem (La Jolla, CA, USA). Proteinase K, tunicamycin and common reagents were from Sigma Chem. Co. (St. Louis, MO, USA).

2.2. Cell lines and culture

Mouse NIH 3T3 fibroblasts expressing H2a (2-18 cells) or H2b (2C cells) were grown in Dulbecco's modified Eagle's medium (DMEM) plus 10% calf serum under 5% CO_2 as described [10].

2.3. Antibodies

A rabbit polyclonal antibody specific for a peptide corresponding to the carboxy-terminus of H2 was the one used in earlier studies [5,10]. A rabbit polyclonal anti-heavy chain binding protein (BiP) was from Stressgen Biotechnologies Corp. (Victoria, BC, Canada).

2.4. Metabolic labeling and immunoprecipitation

Pulse-labeling and chases, immunoprecipitation of H2a and H2b from cell lysates and *N*-glycosidase F treatments were performed as described before [5,10]. For BiP the labeling was done with [^{35}S]cysteine-methionine mix without the addition of unlabeled methionine.

2.5. Gel electrophoresis, fluorography and quantitation

Reducing SDS-PAGE was performed on 10% Laemmli gels except where stated otherwise. For non-reducing conditions β-mercaptoethanol was not included in the buffer. The gels were analyzed by fluorography using 20% 2,5-diphenyloxazole and exposing to BioMax MR film from Eastman-Kodak (Rochester, NY, USA). Quantitation was performed in a Fuji BAS 1000 phosphorimager (Japan).

2.6. Susceptibility to protease digestion

Following metabolic labeling and immunoprecipitation, the immunoprecipitates were washed and the pellets were resuspended in phosphate-buffered saline. Then, proteinase K, at concentrations of 5–50 μg/ml, was added to the resuspended pellets for 15 min on ice. The digestion was stopped by adding 10 mM phenylmethylsulfonyl fluoride, followed by boiling in sample buffer and the samples were subjected to SDS-PAGE.

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Abbreviations: ASGPR, asialoglycoprotein receptor; ALLN, *N*-acetyl-leucyl-leucyl-norleucinal; MG-132, *N*-carbobenzoxyl-leucinyl-leucinyl-leucinal; DTT, dithiothreitol; BiP, heavy chain binding protein; UPR, unfolded protein response

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2.7. In vitro transcription-translation

The reactions were done using an in vitro transcription-translation kit (Promega) according to the manufacturer's instructions. H2a and H2b cDNAs, which are cloned into the *Bam*HI-*Eco*RI sites in the vector pcDNAI, containing the T7 promoter, were linearized with *Eco*RI and cleaned by phenol:chloroform extraction. Then, the linearized plasmids were transcribed with T7 RNA polymerase for 2 h, followed by incubation with RNase-free DNase for 15 min. The nucleotides were removed by passing the RNAs through a Sephadex G50 column and analysis of the RNAs obtained was performed by agarose electrophoresis in the presence of 6.6% formaldehyde. H2a and H2b RNAs were heated for 10 min at 65°C prior to the translation reactions. Then they were translated for 1 h at 30°C using the rabbit reticulocyte cell lysate in the presence of [^{35}S]cysteine-methionine and microsomal membranes. At the end of the translation, the samples were incubated with RNase for 30 min, then boiled for 5 min with sample buffer and subjected to SDS-PAGE, followed by fluorography.

2.8. Protease protection assay

At the end of the in vitro translation, the reactions were transferred to ice and incubated with 0.15 mg/ml of proteinase K for 30 min. Then the samples were precipitated with 10% trichloroacetic acid for 15 min, followed by centrifugation. The pellets were washed with acetone, dried, resuspended in sample buffer and subjected to SDS-PAGE.

3. Results

3.1. ER degradation of H2a does not involve misfolding

Endogenous ASGPR H2a and H2b in HepG2 cells are synthesized as 42 kDa membrane-bound precursors. But whereas H2b is processed in the Golgi to a 50 kDa mature form, H2a precursor is completely retained in the ER unless it is cleaved to a 35 kDa fragment corresponding to its ectodomain which can be secreted [5]. H2a is different from H2b only because it contains an extra juxtamembrane pentapeptide, EGHRG. The different fates of H2a and H2b are reproduced when they are transfected into NIH 3T3 cells where they are normally not expressed. A different degree or rate of folding of H2a compared to H2b could be the cause for the protein to be retained and degraded in the ER. Therefore we analyzed their behavior in an assay that takes advantage of the presence of several disulfide bonds. Newly synthesized membrane and secretory proteins have been shown to migrate as lower mobility bands in non-reducing SDS-PAGE, representing unfolded or partially folded intermediates that are converted into a compact higher mobility band upon folding [11]. Using this assay we had seen that H2a and H2b folded in a similar fashion [6]. However, we could have been looking only at molecules that survived degradation and are thus correctly folded. We therefore incubated cells with a proteasome inhibitor, ALLN [12], and compared their folding (Fig. 1). Cells were labeled with a short pulse of [^{35}S]cysteine followed by chase for 2 h. Before cell lysis the cells were treated with iodoacetamide to block free sulfhydryl groups and after immunoprecipitation the proteins were treated with *N*-glycosidase F to eliminate heterogeneity in the run. As can be seen in Fig. 1, the protein precursors (the region of cleaved fragments is not seen) migrated as unfolded and partially folded species after the pulse (Fig. 1, lane 2); the latter had a fast migration but were sensitive to dithiothreitol (DTT) (lane 3). These bands were converted to a higher mobility band after chase that was mostly resistant to in vivo reduction by DTT as compared to the pulse. The resistance to DTT reflects burial into the protein structure and thus inaccessibility of the disulfide bonds upon folding. ALLN caused mostly accumulation of the folded form for H2a. For H2b there was an increase of both folded and partially folded species; the latter had a fast migration (Fig. 1, lower panel, lane 6) but were partially sensitive to DTT (lane 7). Thus, especially for H2a, most of the molecules that degrade in the absence of ALLN were competent for folding.

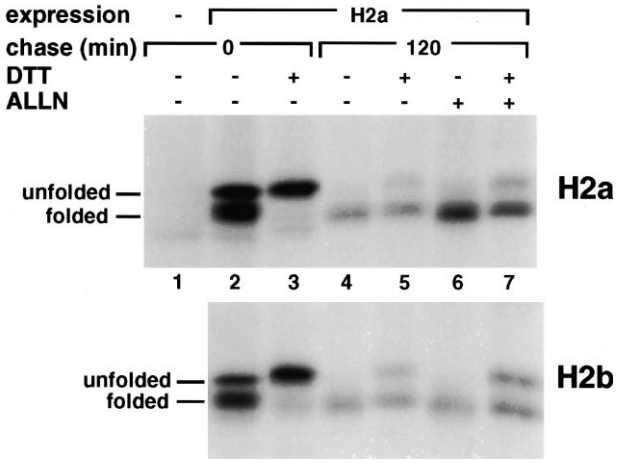

Fig. 1. H2a molecules which are degraded by the proteasome are not unfolded. NIH 3T3 cells (upper panel, lane 1), or the same cells expressing H2a (2-18 cell line, upper panel, lanes 2–7) or H2b (2C cell line, lower panel) were metabolically labeled for 10 min with [^{35}S]cysteine and chased for the indicated times with complete medium in the absence or presence of 100 μM ALLN. In lanes 3, 5 and 7 DTT (5 mM) was added to the cell medium for the last 5 min of the pulse or chase periods. Cells were then incubated with 0.1 M iodoacetamide for 5 min at 4°C, lysed and immunoprecipitated with anti-H2 antibody. The immunoprecipitates were treated with *N*-glycosidase F and run on SDS-PAGE under non-reducing conditions followed by fluorography. Migration of species with reduced 'unfolded' or unreduced disulfide bonds 'folded' is indicated on the left.

To analyze folding using a different assay, independent of disulfide bond formation, we looked at the susceptibility to protease digestion. Unfolded nascent polypeptides are more sensitive to proteolytic cleavage than folded proteins [13]. Incubation of immunoprecipitates of H2a or H2b with proteinase K gave a similar series of small fragments after pulse-labeling (Fig. 2A, lanes 2 and 3). Digestion was almost complete using 50 μg/ml of the protease. After 2 h chase the proteins gave less small fragments. The pattern obtained was very similar for H2a and H2b confirming that the extra H2a pentapeptide does not influence the overall folding of the molecules.

3.2. Expression or accumulation of H2a does not activate the unfolded protein response (UPR)

The UPR is triggered by the presence of misfolded proteins in the ER and involves the upregulation of the expression of genes encoding ER-resident chaperones, e.g. BiP [14]. If H2a were recognized by the cell as a misfolded protein it should trigger the UPR when expressed or even more pronouncedly if these misfolded molecules would accumulate in the presence of proteasome inhibitors. To analyze UPR activation we measured BiP expression by metabolic labeling in 3T3 cells or in the same cells stably expressing H2a and H2b. Only small differences were seen in BiP expression between the different cell lines (Fig. 2B). This was also true even after

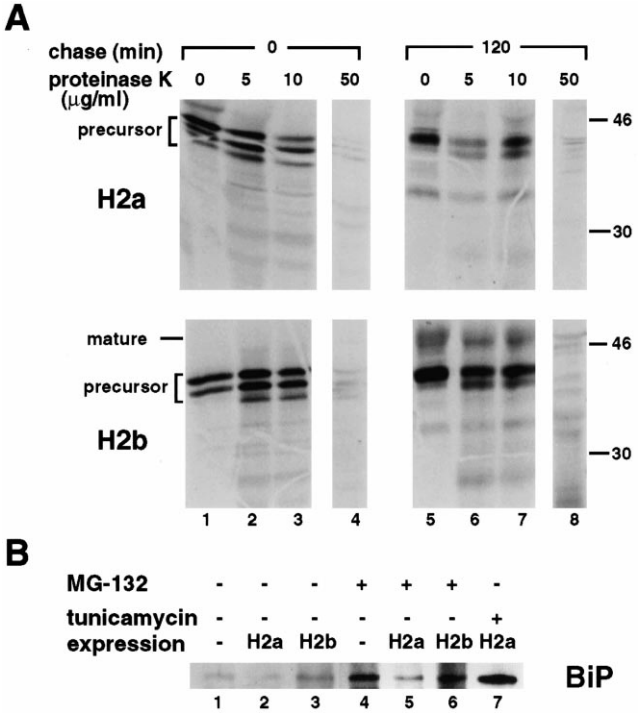

Fig. 2. H2a and H2b show similar folding as judged by susceptibility to proteinase K. Their expression does not induce the UPR. A: Cells expressing H2a or H2b were metabolically labeled for 10 min with [^{35}S]cysteine and chased for 0 or 120 min with complete medium. Cells were then incubated with 0.1 M iodoacetamide for 5 min at 4°C, lysed and immunoprecipitated with anti-H2 antibody. The immunoprecipitates were treated with proteinase K at different concentrations as indicated and analyzed on reducing 12% SDS-PAGE followed by fluorography. On the left is indicated the migration of precursor and Golgi-processed 'mature' molecules. On the right the migration of molecular weight markers in kDa. More than one band is seen for the proteins even without treatment with protease due to heterogeneity of the glycosylation. B: NIH 3T3 cells (lanes 1 and 4) or the same cells expressing H2a (lanes 2, 5 and 7) or H2b (lanes 3 and 6) were preincubated in the absence (lanes 1–3) or presence of either 10 μM MG-132 (lanes 4–6) or 10 μg/ml tunicamycin (lane 7) for 16 h. Cells were then metabolically labeled with [^{35}S]cysteine/methionine for 1 h in the absence or presence of the indicated drugs. Cell lysates were immunoprecipitated with polyclonal rabbit α-BiP antisera. The immunoprecipitates were analyzed by SDS-PAGE followed by fluorography.

treatment of the cells for 16 h with the proteasome inhibitor MG-132, although in this case there was an enhanced expression in all due to a moderate induction of the UPR by the inhibitor itself [15]. Cells expressing H2a show a decreased rather than an increased expression of BiP. As a control to test the ability of cells expressing H2a to produce an UPR they were incubated for 16 h with tunicamycin, a known inducer of the UPR [16], which caused a dramatic increase in BiP levels (Fig. 2B, lane 7).

3.3. Oligomerization of H2a and H2b

Oligomerization was found to be a requirement for the exit from the ER of several proteins [17,18]. When H2a is coexpressed with the ASGPR H1 subunit they do not seem to associate, in contrast to H1 and H2b [5]. This could be a cause for the difference in fate of H2a and H2b when coexpressed with H1 (retention and degradation of H2a versus survival and exit to the Golgi of H2b). This does not, however, explain the ability of H2b to exit the ER when singly expressed as compared to H2a [10]. We therefore analyzed if in the absence of H1 there could be a difference in self-assembly of H2a and H2b. Cells expressing H2a or H2b were metabolically labeled with [^{35}S]cysteine followed by chase for different periods, immunoprecipitation from cell lysates and SDS-PAGE under non-reducing and reducing conditions. The results show the existence of bands that migrate as disulfide-bonded homodimers, trimers and higher oligomers of H2a and H2b under non-reducing conditions (Fig. 3A). However, whereas both H2a and H2b oligomers could be seen in the pulse or after 10 min of chase (Fig. 3A, lanes 2–5), after 2 h of chase only H2b oligomers survived. H2a oligomers

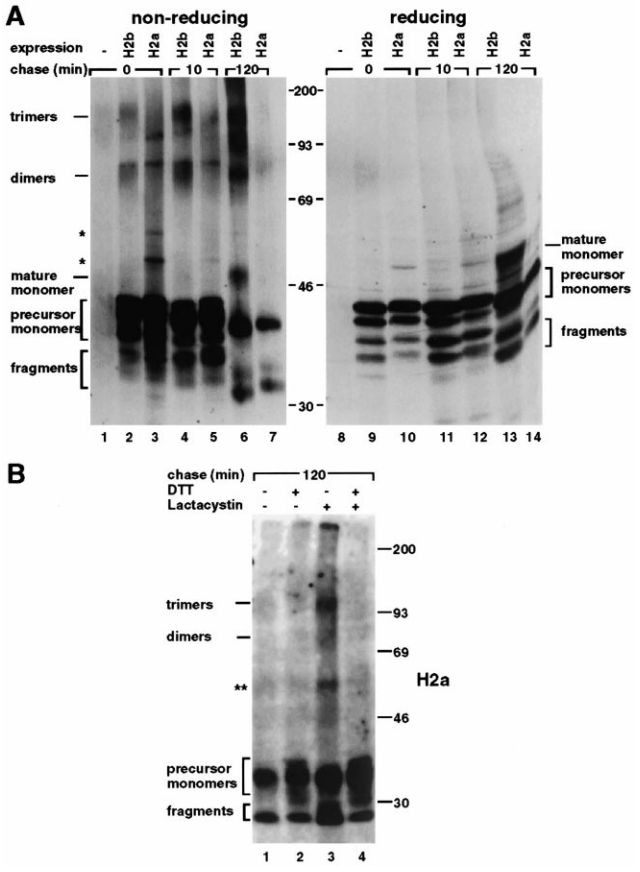

Fig. 3. H2a homooligomers are quickly formed but are then degraded. A: NIH 3T3 cells or the same cells expressing H2a or H2b were metabolically labeled for 10 min with [^{35}S]cysteine and chased for the indicated times with complete medium. Cells were then incubated with 0.1 M iodoacetamide for 5 min at 4°C, lysed and immunoprecipitated with anti-H2 antibody. The immunoprecipitates were run on 8% SDS-PAGE under non-reducing (left panel) or reducing conditions (right panel) followed by fluorography. Migration of precursor or Golgi-processed 'mature' monomers, dimers and trimers and of cleaved soluble fragments is indicated. The asterisks indicate bands that probably represent complexes of H2a with ER-resident proteins. In the reducing gel more than one band is seen for the precursor as well as for the fragment due to varying degrees of occupation of the glycosylation sites. Migration of molecular weight markers is shown in kDa between the two panels. B: An experiment similar to that in A was performed with cells expressing H2a except that immunoprecipitates were treated with *N*-glycosidase F. In lanes 2 and 4, DTT (5 mM) was added to the cell medium for the last 5 min of the chase period. In lanes 3 and 4 lactacystin (10 μM) was added to the cell medium during the chase. The two asterisks indicate a possible dimer of H2a soluble fragment.

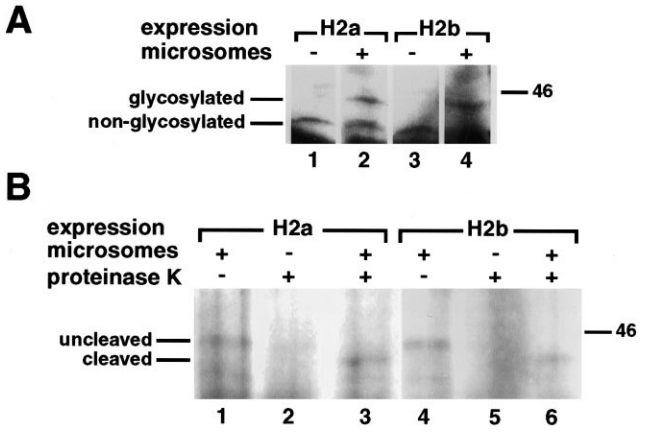

Fig. 4. H2a shows normal translocation into the ER membrane as compared to H2b. A: H2a (lanes 1 and 2) and H2b (lanes 3 and 4) cDNAs were transcribed and translated in vitro, in the absence (lanes 1 and 3) or in the presence (lanes 2 and 4) of microsomal membranes, as described in Section 2. At the end of the translation the samples were boiled in sample buffer for 5 min and subjected to SDS-PAGE followed by fluorography. The glycosylated and non-glycosylated molecules (representing the translocated and non-translocated molecules, respectively) are indicated on the left. On the right the migration of a molecular weight marker in kDa. B: H2a and H2b cDNAs were transcribed and translated in vitro, in the absence (lanes 2 and 5) or in the presence (lanes 1, 3, 4, and 6) of microsomal membranes. In lanes 2, 3, 5, and 6, immediately after the translation, the reactions were incubated with proteinase K (0.15 mg/ml) for 30 min on ice. All the samples were then precipitated with trichloroacetic acid and subjected to SDS-PAGE followed by fluorography. Fragments cleaved by proteinase K or uncleaved are indicated on the left.

were less abundant than those of H2b and disappeared after 2 h chase (Fig. 3A, lanes 6 and 7). After the longer chase period a shift can be seen in the migration of the monomers and fragments under non-reducing conditions due to the more compact structure after folding, as in Fig. 1 (Fig. 3A, lanes 6 and 7). In reducing conditions no oligomers are seen, only bands corresponding to monomers and fragments with different degrees of glycosylation. After 2 h chase a band is seen that corresponds to mature, Golgi-processed H2b (Fig. 3A, lanes 6 and 13), absent for H2a. The degradation of H2a oligomers could be prevented by incubation of the cells with the specific proteasomal inhibitor lactacystin (Fig. 3B, lane 3). In this experiment samples were treated with *N*-glycosidase F to reduce heterogeneity. H2a oligomers that are seen in the presence of lactacystin are S-S-bonded and reduced by incubation of the cells with DTT (Fig. 3B, lane 4). The results show that H2a and H2b can self-assemble but that this does not prevent the ER retention and proteasomal degradation of H2a.

3.4. Similar translocation efficiency for H2a and H2b

Deficient translocation leads to ER retention and degradation of ApoB [8,9] and this could also be true for H2a. To analyze the efficiency of translocation we used an in vitro transcription-translation assay with cDNAs encoding H2a or H2b. The proteins were synthesized in a reticulocyte lysate in the presence or absence of dog pancreas microsomes. Both H2a and H2b showed a similar shift in molecular weight in the presence of the microsomes that represents glycosylation of the three *N*-glycosylation sites (Fig. 4A). This indicates complete translocation of the carboxy-terminal ectodomain into the ER lumen. This was confirmed by incubation of the samples with proteinase K, which cleaved the short cytoplasmic tail of both H2a and H2b in the presence of the microsomes to yield fragments of the same size (Fig. 4B, lanes 3 and 6 compared to 1 and 4). In contrast, proteinase K degraded the proteins completely without the protection of the microsomal membranes (Fig. 4B, lanes 2 and 5).

4. Discussion

One of the best known causes for ER retention and degradation is protein malfolding. Several mutated proteins like the α1 antitrypsin Z mutant or the cystic fibrosis transmembrane conductance regulator mutant ΔF508 have been shown to be misfolded, leading to their retention in the ER and degradation through the cytosolic ubiquitin/proteasome machinery [19–21]. The extra charged pentapeptide EGHRG that occurs naturally in ASGPR H2a and not in its variant H2b was shown to cause the ER retention of the H2a membrane-bound precursor. When the pentapeptide was introduced into the sequence of the H1 subunit it caused its total retention in the ER but this effect was not achieved by causing a general misfolding of H1 [6]. As shown in Figs. 1 and 2 the presence of the pentapeptide in H2a precursor does not trigger malfolding of the protein which could have been a cause for its ability to serve as a substrate of the ER quality control machinery. Folding was analyzed in two different ways, one based on the presence of eight cysteine residues able to form disulfide bonds in the ectodomain of H2a and H2b [22]. The progressive formation of the disulfide bonds is accompanied by their burying into the compact structure of the molecule during its folding and renders them resistant to unfolding in vivo by DTT (Fig. 1). The second assay is based on the progressive resistance to protease digestion during folding of the molecules (Fig. 2A). In both assays H2a showed a very similar behavior to H2b indicating a similar general pattern and kinetics of folding of the molecules. No significant aggregation of H2a molecules, or presence of Triton-insoluble forms was observed either (data not shown), in contrast to that seen for several misfolded proteins [21,23]. Inhibition of the degradation of H2a by proteasomal inhibitors led to the accumulation of folded rather than unfolded forms (Fig. 1), indicating that folding competent molecules can also be degraded by the proteasome. After a lag these molecules would be tagged, perhaps by ubiquitination on their cytosolic tails or by association with chaperones, and then retrotranslocated to the proteasome. The translocation channel (Sec61 complex) is large enough to accommodate folded H2a monomers. Unfolding prior to degradation might be accomplished by ATPases at the 19S subunit of the proteasome [24]. The proteasomal degradative activity may be coupled to the unfolding process and therefore inhibition of the degradation would also block the unfolding.

Proteasomal inhibitors did not inhibit the cleavage and secretion of the H2a fragment, secretion was in fact increased in their presence (Kamhi and Lederkremer, unpublished). In contrast to the complete ER retention of H2a precursor, about 30% of singly expressed H2b exits to the Golgi, the rest being degraded [10]. When the degradation of H2b was inhibited, a small increase in the ratio of partially folded to folded molecules was observed, from 34% of partially folded molecules in the absence of the inhibitor to 48% in its pres-

ence, indicating that at least part of the degradation of H2b may be caused by incomplete folding of the molecules, in agreement with a previous report [25]. Fig. 2B shows that the accumulation of H2a or even of partially folded H2b does not trigger the UPR, which arises from the accumulation of truly misfolded proteins [14].

Assembly of most oligomeric proteins occurs prior to their exit from the ER. In fact it is generally a prerequisite for exit from the ER of many proteins. This is for instance the case with the immunoglobulins [26,27], MHC class I [28], and the T cell antigen receptor [17,29]. The ASGPR assembles into a heterooligomer of H1 and H2b subunits. Singly expressed H2b matures to the cell surface with an efficiency of about 30%, but co-expression with the H1 subunit results in a significant increase in its maturation through the Golgi. On the other hand H2a precursor or cleaved fragment do not seem to associate with H1 [5]. In the absence of the H1 subunit both H2a and H2b showed a significant level of self-assembly (Fig. 3). Homodimerization was shown to be a mechanism for exit from the ER, as for example in the case of immunoglobulin light chains expressed without the heavy chains [30]. The H2 oligomers are disulfide-bonded as they cannot be seen in reducing conditions (Fig. 3). However, while the H2b oligomers were stable those of H2a were totally degraded after a chase period and this degradation could be inhibited with a proteasomal inhibitor (Fig. 3B). Therefore self-assembly of H2a precursor does not prevent its total ER retention and degradation. Other cases have been reported where oligomerization does not override ER retention, like the case of the gp80 complex mutants that can form homooligomers in the absence of the second subunit [31]. Insulin receptor monomers can still very efficiently dimerize in the absence of interaction with the chaperones calnexin and calreticulin but these abnormal dimers are retained in the ER [32]. In the case of H2a, its precursor is retained in the ER due to the pentapeptide retention signal and this retention cannot be precluded by self-assembly. As the retention is followed by proteasomal degradation future research should address the possible existence of a step of disassembly of the oligomers prior to retrotranslocation to the cytosol.

The EGHRG-charged juxtamembrane sequence could have altered the efficiency of translocation or even the orientation of H2a as this is determined by the difference in charges of the membrane-flanking segments [33]. Altered translocation can cause ubiquitination and ER degradation as demonstrated for ApoB [34]. However, in an in vitro transcription-translation assay H2a and H2b showed a similar translocation into the microsomal lumen (Fig. 4). Only the small amino-terminal domain of H2a and H2b was still accessible for digestion on the cytoplasmic face of the microsomes (Fig. 4B). This confirms the complete translocation and correct type II transmembrane orientation of the proteins.

In conclusion, the complete ER retention and degradation of H2a precursor are not caused by incorrect translocation nor prevented by folding or self-assembly. The retention is a consequence of the presence of the retention determinant EGHRG and degradation occurs when after a reasonable time the precursor is not cleaved to its mature soluble form. In fact, there is a lag of about 1 h before degradation occurs; the end of this lag might be signaled by glucose and mannose trimming reactions on H2a *N*-linked oligosaccharides [35].

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References

[1] Hammond, C. and Helenius, A. (1995) Curr. Opin. Cell Biol. 7, 523–529.
[2] Kopito, R.R. (1997) Cell 88, 427–430.
[3] Bonifacino, J.S. and Weissman, A.M. (1998) Annu. Rev. Cell Dev. Biol. 14, 19–57.
[4] Wilbourn, B., Nesbeth, D.N., Wainwright, L.J. and Field, M.C. (1998) Biochem. J. 111–118.
[5] Tolchinsky, S., Yuk, M.H., Ayalon, M., Lodish, H.F. and Lederkremer, G.Z. (1996) J. Biol. Chem. 271, 14496–14503.
[6] Shenkman, M., Ayalon, M. and Lederkremer, G.Z. (1997) Proc. Natl. Acad. Sci. USA 94, 11363–11368.
[7] Field, M.C., Moran, P., Li, W., Keller, G.A. and Caras, I.W. (1994) J. Biol. Chem. 269, 10830–10837.
[8] Liang, J. et al. (1998) J. Biol. Chem. 273, 35216–35221.
[9] Macri, J. and Adeli, K. (1997) J. Biol. Chem. 272, 7328–7337.
[10] Lederkremer, G.Z. and Lodish, H.F. (1991) J. Biol. Chem. 266, 1237–1244.
[11] Braakman, I., Helenius, J. and Helenius, A. (1992) Nature 356, 260–262.
[12] Neumann, D., Yuk, M.H., Lodish, H.F. and Lederkremer, Z. (1996) Biochem. J. 313, 391–399.
[13] Wada, I., Ou, W.J., Liu, M.C. and Scheele, G. (1994) J. Biol. Chem. 269, 7464–7472.
[14] Sidrauski, C. and Walter, P. (1997) Cell 90, 1031–1039.
[15] Bush, K.T., Goldberg, A.L. and Nigam, S.K. (1997) J. Biol. Chem. 272, 9086–9092.
[16] Pahl, H.L. and Baeuerle, P.A. (1995) EMBO J. 14, 2580–2588.
[17] Bonifacino, J.S., Suzuki, C.K., Lippincott-Schwartz, J., Weissman, A.M. and Klausner, R.D. (1989) J. Cell Biol. 109, 73–83.
[18] Gardner, A.M., Aviel, S. and Argon, Y. (1993) J. Biol. Chem. 268, 25940–25947.
[19] Qu, D.F., Teckman, J.H., Omura, S. and Perlmutter, D.H. (1996) J. Biol. Chem. 271, 22791–22795.
[20] Sommer, T. and Wolf, D.H. (1997) FASEB J. 11, 1227–1233.
[21] Ward, C.L., Omura, S. and Kopito, R.R. (1995) Cell 83, 121–127.
[22] Yuk, M.H. and Lodish, H.F. (1993) J. Cell Biol. 123, 1735–1749.
[23] Marquardt, T. and Helenius, A. (1992) J. Cell Biol. 117, 505–513.
[24] Hochstrasser, M. (1995) Curr. Opin. Cell Biol. 7, 215–223.
[25] Wikstrom, L. and Lodish, H.F. (1993) J. Biol. Chem. 268, 14412–14416.
[26] Shachar, I., Amitay, R., Rabinovich, E., Haimovich, J. and Bar, N.S. (1992) J. Biol. Chem. 267, 24241–24247.
[27] Fra, A.M., Fagioli, C., Finazzi, D., Sitia, R. and Alberini, C.M. (1993) EMBO J. 12, 4755–4761.
[28] Ortmann, B., Androlewicz, M.J. and Cresswell, P. (1994) Nature 368, 864–867.
[29] Wileman, T., Kane, L.P., Young, J., Carson, G.R. and Terhorst, C. (1993) J. Cell Biol. 122, 67–78.
[30] Leitzgen, K., Knittler, M.R. and Haas, I.G. (1997) J. Biol. Chem. 272, 3117–3123.
[31] Losch, A., Henkel, K., Wagner, M., Urban, J. and Kochbrandt, C. (1996) Eur. J. Cell Biol. 69, 107–115.
[32] Bass, J., Chiu, G., Argon, Y. and Steiner, D.F. (1998) J. Cell Biol. 141, 637–646.
[33] Hartmann, E., Rapoport, T.A. and Lodish, H.F. (1989) Proc. Natl. Acad. Sci. USA 86, 5786–5790.
[34] Chen, Y., Le, C.F. and Chuck, S.L. (1998) J. Biol. Chem. 273, 11887–11894.
[35] Ayalon-Soffer, M., Shenkman, M. and Lederkremer, G.Z. (1999) J. Cell Sci. (in press).