Biochemical and Biophysical Research Communications 451 (2014) 361–366

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# The human ubiquitin conjugating enzyme UBE2J2 (Ubc6) is a substrate for proteasomal degradation

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ARTICLE INFO

*Article history:*
Received 18 July 2014
Available online 30 July 2014

*Keywords:*
Unfolded protein response (UPR)
ER associated degradation (ERAD)
Ubiquitin conjugating enzyme
Ube2J2 (Ubc6)
Ube2G2 (Ubc7)

ABSTRACT

The human Ube2J2 enzyme functions in the ubiquitination of proteins at the ER. Here we demonstrate that it, and a second ubiquitin conjugating (Ubc) enzyme Ube2G2, are unstable, and incubation of transfected cells with proteasome inhibitors increased steady-state protein levels. For Ube2J2, pharmacological induction of the unfolded protein response (UPR) did not significantly alter ectopic protein levels, however the effect of proteasomal inhibition was abolished if the enzyme was inactivated or truncated to disrupt its ER-localization. These results suggest for the first time that the steady state expression of Ubcs' may be important in regulating the degradation of ER proteins in mammalian cells.



## 1. Introduction

The poly-ubiquitination of proteins, which precludes their degradation by the 26S proteasome, involves the activity of a number of cellular enzymes [1]. Ubiquitin is initially activated in an ATP-dependent manner by an E1 activating enzyme before it is transferred to a conserved cysteine residue located within the active site of an E2 ubiquitin conjugating enzyme (Ubc) [2]. The next step in the ubiquitination cascade is catalyzed by E3 ubiquitin-protein ligases that either directly (HECT E3s') or indirectly (RING E3s') mediate the transfer of ubiquitin from the Ubc enzyme onto the target substrate [3].

The majority of secretory and membrane proteins are synthesized and folded correctly at the endoplasmic reticulum (ER). However, an increasing number of genetic and environmental conditions have been described where the persistent misfolding of ER transiting proteins can occur [4]. When this happens it results in the induction of an evolutionarily conserved cellular stress pathway called the unfolded protein response (UPR), where the activation of sensory proteins PERK, ATF6 and Ire1 promotes downstream signaling events to restore homeostasis and protect the cell from proteotoxic stress [5].

ER associated degradation (ERAD) of misfolded proteins by the ubiquitin proteasome pathway is a key component of the UPR, and a number of ER-localizing E2 and E3 enzymes have been described [6]. Ube2J2 (Ubc6) is one such ER-localized E2 enzyme [7,8], and Northern blot analysis shows that increases in transcription of the gene can be detected within 4 h of UPR induction [9]. The protein has been shown to act in association with a number of different E3 enzymes including TEB4 [10], parkin [11], CHIP [12] and cIAP1 [13]. Together, these data suggest that Ube2J2 plays a varied and important role in proteasomal degradation at the ER.

The carboxyl terminus of Ube2J2 contains a hydrophobic region that has been hypothesized to mediate insertion of the protein into the ER membrane [7,8]. A recent study has demonstrated that this region of the protein can promote proteasomal degradation when expressed as part of a fusion protein [14] however it was not clear whether the Ube2J2 protein is unstable in its own right. Here we specifically addressed this question and for the first time describe the role played by ER localization and enzymatic activity in regulating steady state expression of the human Ube2J2 protein.

*Abbreviations:* Ubc, ubiquitin conjugating enzyme; UPR, unfolded protein response; E3, ubiquitin protein ligase.

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http://dx.doi.org/10.1016/j.bbrc.2014.07.099
0006-291X/

## 2. Materials and methods

### 2.1. Plasmid DNA and siRNA's

The coding sequences for human Ube2J2 and Ube2G2 were PCR amplified from a human testes cDNA library and cloned into the pEP7-HA vector [15]. Full-length and truncated Ube2J2 (encoding amino acid residues 1–227 and therefore lacking the carboxyl-terminus hydrophobic region – ΔTM) were cloned into the pEP7-GFP or pEP7-FL vectors [16]. The QuickChange protocol (Stratagene) was used to mutate active site cysteine residues C94 and C89 of the Ube2J2 and Ube2G2 proteins respectively to serines. Co-transfection of HFE-HA and β2microglobulin-FL plasmids have previously been described [17]. Control and Ube2J2 specific siRNA (Sense, 5′ → 3′, GCACAAGACGAACUCAGUAtt) were purchased from Ambion.

### 2.2. Cell culture and transfections

HEK293T and HeLa cells (ATCC) were seeded at densities of either $0.75 \times 10^5$ cells per well on 24 well plates or $10 \times 10^5$ on 100 mm dishes and transfected with Lipofectamine 2000 as described elsewhere [18]. Cells were incubated in the presence or absence of pharmacological agents purchased from Calbiochem (lactacystin) or Sigma (MG132, tunicamycin, thapsigargin, DTT) as described in figure legends. Cells were harvested in RIPA buffer as described elsewhere [17]. For cell fractionation studies whole cell lysates harvested and sonicated in 0.1 M sodium phosphate buffer (pH7.4) were ultracentrifuged at 120,000 rpm in a RM130 rotor in a Beckmann benchtop ultracentrifuge for 1h at 4 °C. Supernatants were removed, and washed pellets were resuspended in Laemmli buffer before gel electrophoresis.

### 2.3. Immunoblot analysis

Lysates were fractionated on denaturing 10% SDS–polyacrylamide gels for immunoblotting by standard methods with mouse anti-FLAG (Sigma M2-1:1000), rabbit anti-BiP (CellSignalling-1:1000), mouse anti-HA (Covance-1:1000) or rabbit anti-Ube2J2 (Davids Biotechnology-1:150) antibodies. Equal loading of gels was confirmed using a mouse anti-β-actin antibody (Sigma). All immunoblots shown are representative of at least 3 independent experiments. Immunoblot imaging and densitometric analysis were performed on a LiCor Odyssey imager. Statistical analysis was performed using a Students paired *t*-test.

### 2.4. Fluorescence imaging

HEK293T cells were transfected to express the pEP7-Ube2J2-GFP and pEP7-Ube2J2ΔTM-GFP vectors. Ten minutes before harvesting 1.5 μM RED-ER tracker (Invitrogen) was added before fixing for 30 min in 5% paraformaldehyde and mounting in Mowiol. Fluorescence was localized within the cell using either Leica inverted or Zeiss LSM510META confocal microscopes.

## 3. Results

### 3.1. The human Ube2J2 enzyme is a substrate for proteasomal degradation

Recent studies concluded that the hydrophobic tail of Ube2J2 promotes proteasomal degradation when expressed at the carboxyl end of a chimeric fusion protein [14] but provided little insight into how steady state expression of the Ube2J2 protein itself might be regulated. To further our understanding of human Ube2J2 therefore, and specifically explore whether the proteasome is involved in regulating its expression, we incubated HeLa cells in the presence or absence of the proteasome inhibitor lactacystin. As can be seen in Fig. 1A, this treatment led to an increase in the expression of an endogenous protein of the expected 28 kDa size (2.2 ± 0.4-fold, mean ± SEM, $n = 3$). To confirm that this represented Ube2J2 we used validated siRNAs (Fig. 1B) to demonstrate that the 28 kDa protein was knocked down by Ube2J2 specific siRNA (Fig. 1C).

These data demonstrated for the first time that the proteasome might play a role in regulating Ube2J2 protein expression. To confirm this independently we transfected HEK293T cells to express Ube2J2-HA and incubated the cells in the presence or absence of lactacystin. As can be seen in Fig. 1D, this resulted in an increase in steady state expression (2.0 ± 0.3-fold, mean ± SEM, $n = 3$) suggesting that the regulation of ectopic Ube2J2 expression in transfected cells is comparable to that observed for the endogenous protein. Similar results were obtained in experiments where the proteasome inhibitor MG132 was used (2.1 ± 0.3-fold, mean ± SEM, $n = 3$, data not shown). Not only is the human Ube2J2 protein involved in mediating the degradation of ER proteins therefore, but it also appears to be a target for proteasomal degradation in its own right.

### 3.2. Steady state expression of Ube2J2 is not regulated by UPR induction

Given the central role that Ube2J2 is reported to play in the UPR and ERAD [7,8] we next wondered if UPR induction can regulate steady state expression of the protein. In order to test this we incubated Ube2J2 transfected cells for 16 h with UPR-inducing drugs. Increases in expression of the ER chaperone BiP confirmed the induction of ER stress following treatment with thapsigargin (Thaps.), tunicamycin (Tun.) and DTT (Fig. 2A, upper panel and Fig. 2B). Despite UPR induction, we could not detect any increases in ectopic Ube2J2 protein levels (Fig. 2A, middle panel and Fig. 2B).

Changes in the steady state expression of proteins can frequently be detected over shorter time intervals. To assess if this might be the case for Ube2J2 we performed tunicamycin experiments over a reduced time course, with the glycoprotein HFE-HA [17] confirming the accumulation of unglycosylated protein in tunicamycin treated samples. Despite the shorter time course we once again failed to detect significant increases in ectopic Ube2J2 expression (Fig. 2C).

### 3.3. Proteasomal degradation of Ube2J2 is associated with localization to the ER

The hydrophobic carboxyl-terminus of human Ube2J2 has been shown to promote proteasomal degradation when expressed at the carboxyl end of a fusion protein [14]. Deletion of this same carboxyl-terminus region disrupts the post-translational association of *in vitro* translated protein with canine microsomal membranes, leading to proposals that this region mediates cellular ER localization of the protein [8]. We wondered whether the proteasomal degradation of Ube2J2 that we observed might be occurring specifically as a result of its ER localization. In order to test this we generated plasmids that express full-length or truncated (lacking the hydrophobic region) Ube2J2-GFP chimeras. As can be seen in the upper panels of Fig. 3A the full-length protein co-localized with ER-tracker in our transfected HEK293T cell model. In contrast, we observed that expression of the truncated Ube2J2ΔTM-GFP protein was not restricted to the ER (Fig. 3A lower panels) and despite the fact that equal quantities of plasmid were transfected during these experiments, it was noted that the truncated protein appeared to be present at higher levels (compare upper and lower left hand

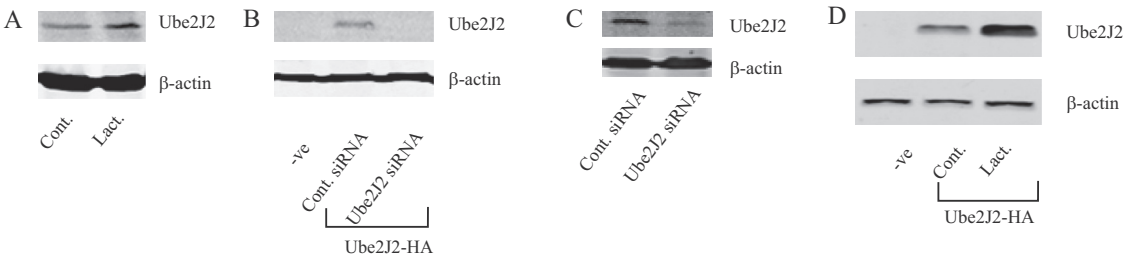


**Fig. 1.** Human Ube2J2 is a substrate for proteasomal degradation. (A) HeLa cells were incubated in the presence or absence of 10 μM lactacystin (Lact.) for 4 h before harvesting and anti-Ube2J2 immuno-blotting. (B) HeLa cells were transfected to express Ube2J2-HA and co-transfected with control or Ube2J2 specific siRNA. Cells were harvested for detection of Ube2J2 in anti-HA immunoblots. (C) HeLa cells were transfected with control or Ube2J2 specific siRNA. Cells were harvested and lysates analyzed in anti-Ube2J2 immunoblots. (D) HEK293T cells transfected to express Ube2J2-HA were incubated in the presence or absence of 10 μM lactacystin for 4 h before harvesting and analysis by anti-HA immunoblotting.

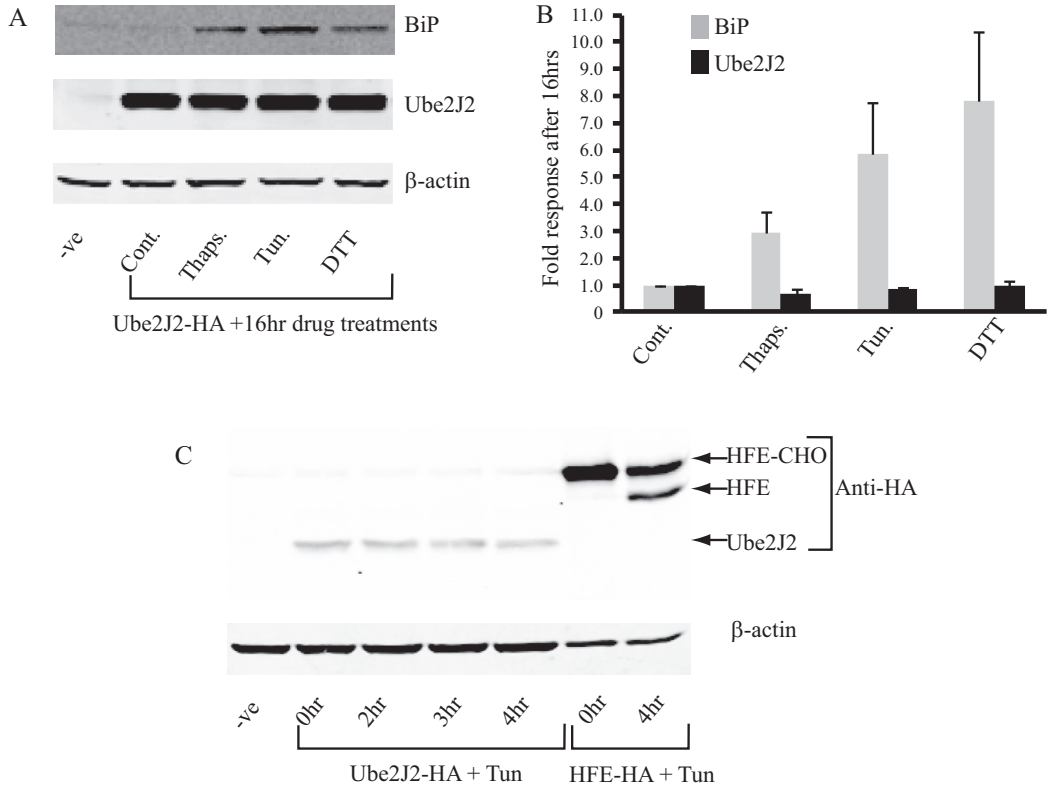


**Fig. 2.** UPR induction does not alter steady state expression of Ube2J2. (A) HEK293T cells transfected to express Ube2J2-HA were incubated for 16 h with 2 μg/ml tunicamycin, 3 μM thapsigargin, or 1 mM DTT. BiP and HA-tagged protein expression was analyzed by immuno-blotting. (B) Graph of fold changes in BiP or Ube2J2-HA protein levels compared to controls and normalized for β-actin expression levels as described in (A) (mean ±SEM, $n = 3$). (C) HEK293T cells transfected to express Ube2J2-HA or HFE-HA/β2M-FL were incubated with 2 μg/ml tunicamycin for the indicated times. HA-tagged protein expression was analyzed by anti-HA immuno-blotting. For lysates containing HFE-HA one third of the total protein was loaded compared to Ube2J2-HA containing lysates to compensate for differences in the steady state expression of the two proteins. HFE and HFE-CHO refer to the deglycosylated and glycosylated forms of HFE respectively.

panels of Fig. 3A). This provided a preliminary indication that disruption of ER localization might indeed be influencing Ube2J2 stability. To further explore this we incubated cells expressing Ube2J2-FL or Ube2J2ΔTM-FL with lactacystin. As can be seen in Fig. 3B the truncated protein was insensitive to proteasomal inhibition compared to the full-length.

These data indicated that ER localization of Ube2J2 influences its proteasomal degradation and it remained a possibility that increases in expression resulting from lactacystin treatment (such as in Fig. 1 for example) could reflect an indirect effect resulting from re-localization. As can be seen in Fig. 3C however, lactacystin treatment does not dramatically alter the pattern of Ube2J2-GFP localization within the cell and increases in Ube2J2 levels associated with proteasomal inhibition could be detected specifically in the membrane fraction (Fig. 3D). These combined data suggested that lactacystin treatment acts to inhibit Ube2J2 degradation at the ER (ERAD), as opposed to disrupting its ER localization.

### *3.4. Catalytically important residue cysteine C94, is important for the proteasomal degradation of Ube2J2*

A number of mammalian E3 ubiquitin protein ligases have been shown to mediate their own proteasomal degradation. This can be demonstrated experimentally by catalytic inactivation of the enzymes through mutagenesis and observing an increase in steady state expression [3]. A conserved cysteine residue in the active site

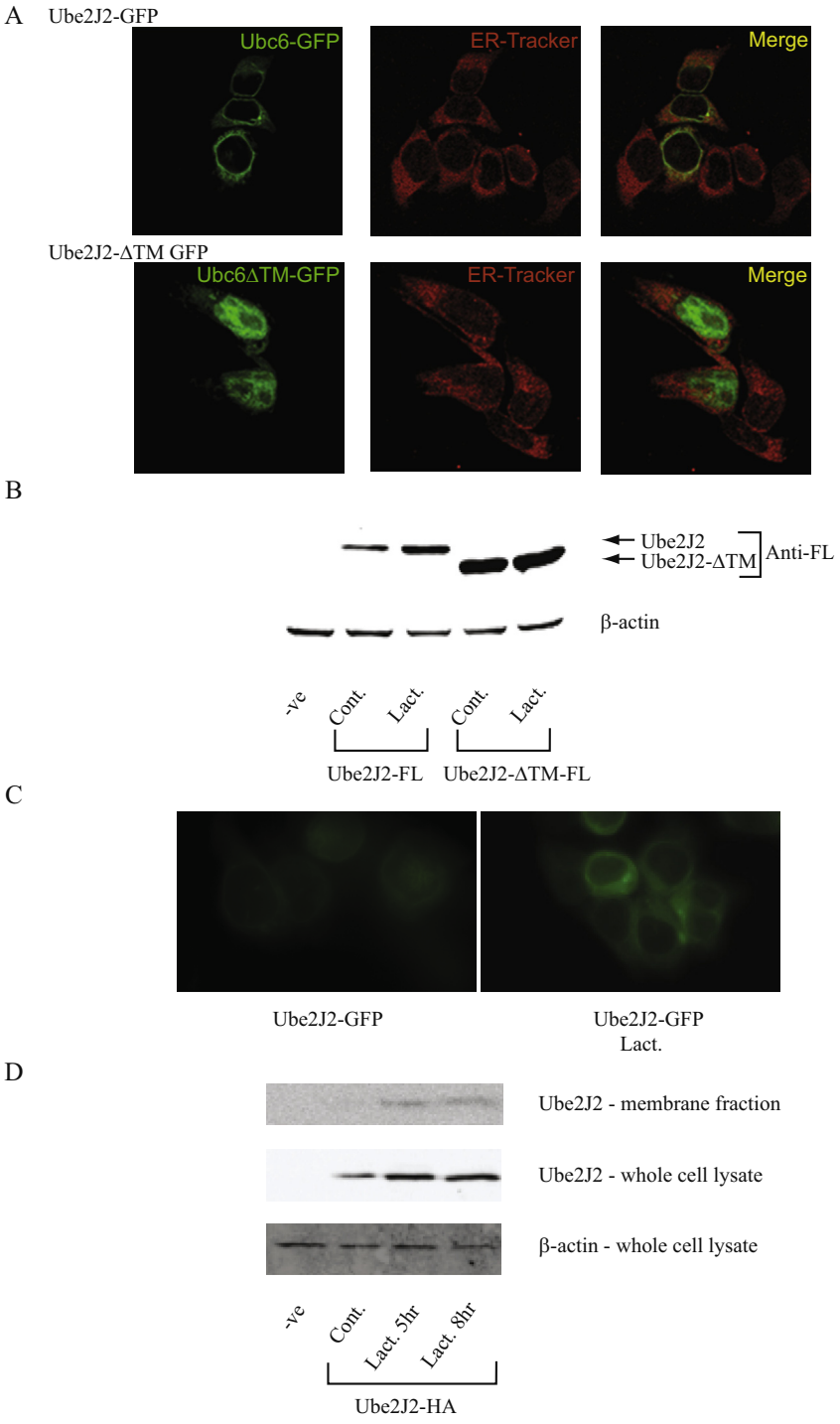


**Fig. 3.** Proteasomal degradation of Ube2J2 is associated with localization to the ER (A) HEK293T cells were transfected to express Ube2J2-GFP or Ube2J2-ΔTM-GFP as indicated. Confocal microscopy using identical fluorescence detection settings was used to localize cellular expression of fluorescent proteins by comparison with a commercially available ER-tracker dye. (B) HEK293T cells transfected to express FLAG-tagged Ube2J2-FL or Ube2J2-ΔTM-FL were incubated with 10 μM lactacystin (Lact.) for 4 h as indicated and cell lysates were analyzed by anti-FL immuno-blotting. (C) HEK293T cells transfected to express Ube2J2-GFP were incubated with 10 μM lactacystin (Lact.) for 6 h. Fluorescence microscopy using identical fluorescence detection settings was used to examine the cellular expression of Ube2J2 in response to 10 μM lactacystin treatment. (D) HEK293T cells transfected to express Ube2J2-HA were incubated in the presence of 10 μM lactacystin (Lact.) for the indicated time periods. HA-tagged protein expression was analyzed by anti-HA immunoblots of whole cell lysates (WCL), or membrane (Memb.) fractions following untracentrifugation.

of all Ubc enzymes mediates the catalytic reaction [2]. To test for whether human Ube2J2 plays a role in its own degradation the active site cysteine residue C94 was mutated to serine, and HEK293T cells were transfected with equal quantities of plasmid DNA to express either the wild type Ube2J2-HA or Ube2J2-HA$^{C94S}$ mutant proteins. 48 h later whole cell lysates were analyzed for protein expression. Results from these studies demonstrated that the active site mutation was leading to increases in the steady state expression of Ube2J2 (Fig. 4A).

We wished to examine whether this increase in expression was a reflection of decreased degradation specifically by the proteasome, and cells transfected to express the inactive Ube2J2-HA$^{C94S}$

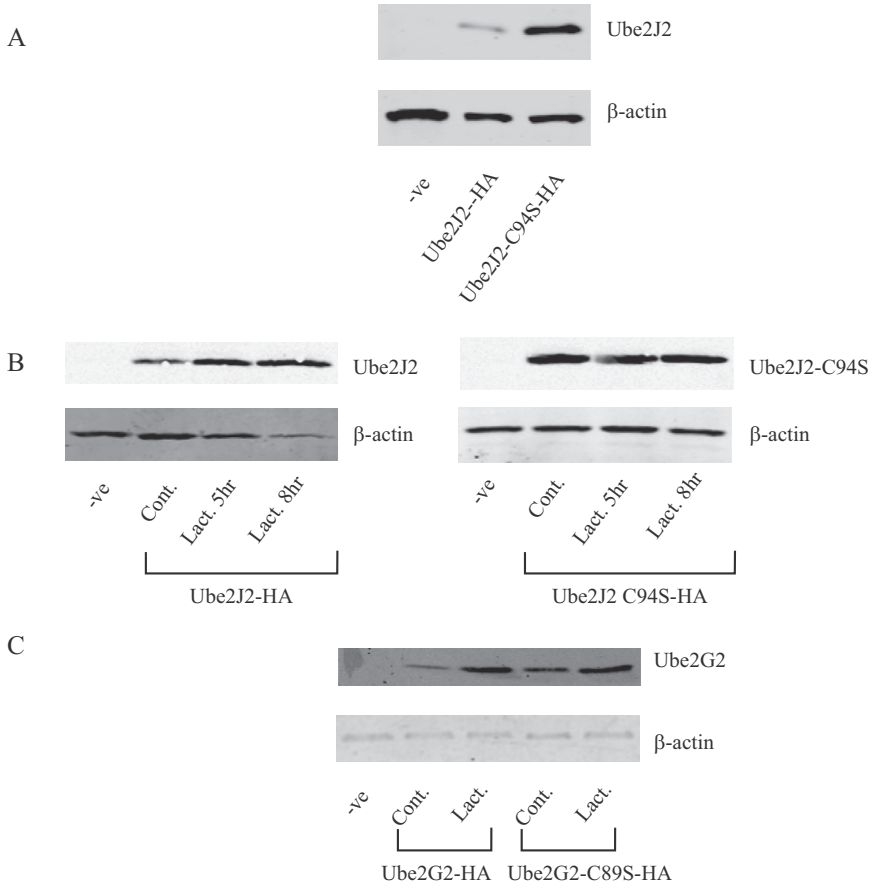


**Fig. 4.** Proteasomal degradation of Ube2J2 is associated with catalytic activity of the enzyme itself. (A) HEK293T cells transfected to express Ube2J2-HA or Ube2J2-HA$^{C94S}$ isoforms were analyzed for HA-tagged protein expression in anti-HA immunoblots. (B) HEK293T cells transfected to express Ube2J2-HA (left panel) or mutant Ube2J2-HA$^{C94S}$ (right panel) were incubated with 10 μM lactacystin (Lact.) for the indicated times and analyzed by anti-HA immunoblots. (C) Transfected HEK293T cells expressing Ube2G2-HA or Ube2G2-HA$^{C89S}$ were incubated of lactacystin (10 μM) for 5 h. Lysates were analyzed with anti-HA immunoblots.

enzyme were incubated in the presence or absence of lactacystin. As is clear from the immunoblots in Fig. 4B the mutant isoform was no longer sensitive to proteasomal degradation (right hand panel) when compared to the wild type protein (left hand panel) in paired experimental samples.

We next wondered whether the patterns of protein expression described above are restricted to Ube2J2, or whether the human Ube2G2 enzyme that also participates in the degradation of proteins at the ER might be similarly regulated. To examine this question HEK293T cells were transfected to express Ube2G2-HA. As can be seen in Fig. 4C, human Ube2G2, like Ube2J2, is a target for proteasomal degradation, and following lactacystin treatment an increase in expression was detected in whole cell lysates. When cysteine residue C89 in the Ube2G2 active site was mutated to serine however (Fig. 4C) the mutant isoform reproducibly retained some sensitivity to lactacystin treatment suggesting that additional factors are important for its degradation.

## 4. Discussion

UPR signaling leads to the activation of transcription factors and increased transcription of ERAD component genes that can reduce ER stress [19]. There is evidence in recent years however that ERAD output can also be controlled post-transcriptionally by influencing the steady state expression of ERAD components [20]. Here we demonstrate that the human Ube2J2 protein, expressed endogenously in HeLa cells or ectopically in HEK293T cells, is subject to proteasomal degradation. It therefore joins an ever increasing number of ERAD components that can be regulated in this way [21]. However, unlike some of these other components whose steady state expression can be increased by UPR signaling [21], we found that ectopic protein levels were not altered after treatment with drugs that are known to induce ER stress. This applied not only to overnight 16 h drug treatments but also to shorter 4 h treatments where we observed the accumulation of de-glycosylation forms of HFE as a positive control for tunicamycin treatment. UPR conditions will nevertheless be associated with higher levels of Ube2J2 as a result of increased gene transcription [9], however our data suggests that it does not increase the stability of the protein.

Our data complement those of Claessen et al. [14] who showed that the tail of Ube2J2 can in its own right promote the proteasomal degradation of a fusion protein. Here we showed that removing this region, and the resulting disruption of ER localization, abrogates sensitivity to proteasome inhibition. The mechanism by which Ube2J2 gets targeted to the ER membrane has not yet been established, however our data suggests that this could potentially be a point for regulating cellular levels of the protein. There is precedence for this type of regulation, as it has been shown that ER-localization of the SGK1 protein specifically facilitates its proteasomal degradation, while regulated disruption of this localization leads to protein stabilization [22]. Other proteins, which like Ube2J2 have a hydrophobic carboxyl anchor region, are known to utilize ASNA1 and WRB to facilitate ER insertion [23]. However it is not yet clear if these mediate Ube2J2 insertion in the membrane,

or if this mechanism can be regulated so as to influence the stability of ERAD substrates.

From an evolutionary perspective the mechanism of ERAD is highly conserved across eukaryotic species. However, compared to yeast where the response is the best described, studies are continually highlighting areas of divergence with mammals, with many additional levels of complexity being observed (in humans for example two differentially regulated E3 enzyme homologs exist for the single yeast Hrd1p enzyme [24]). In our studies we highlight both similarities and divergence compared to the yeast enzymes that show greatest homology to Ube2J2 (scUbc6p – approximately 40% identity) and Ube2G2 (scUbc7p – approximately 60% identity). Our studies show that Ube2J2, like its yeast homolog, is involved in regulating its own proteasomal degradation [25]. Attempts to characterize this in yeast have focused on the Doa10p E3 enzyme, which, in addition to interacting functionally with Ubc6p, has also now been shown to mediate its degradation [26]. Ube2J2 functionally interacts with a number of E3 enzymes however, and additional studies will be required to characterize whether any of these are involved in regulating the steady state levels of the protein.

On the other hand we noted divergence in the regulation of Ube2G2 and its yeast homolog Ubc7p. Specifically, while polyubiquitination of the active site cysteine is critical for the degradation of Ubc7p [27], we noted that our active site Ube2G2-C89S mutant retained some degree of lactacystin sensitivity, suggesting that additional factors contribute towards its proteasomal degradation. The Ube2G2 protein, in contrast to UIbe2J2, is not restricted to the ER membrane however and further analysis will need to address whether the cytosolic and ER pools are differentially regulated in mammalian cells.

In conclusion, our results demonstrate that ER localization and *cis*-enzymatic activity play a role in regulating the steady state expression of human Ube2J2, and suggest for the first time that the steady state expression of mammalian ERAD Ubc enzymes may be of importance for the effective degradation of ER transiting proteins.

## Acknowledgments

This work was supported by the Portuguese Fundação para Ciência e a Technologia (POCI/SAU-MMO/61129/2004) and the Irish Health Research Board (RP/2006/294) to J.V.F., L.A.F. is a recipient of an Irish Research Council for Science Engineering and Technology studentship. S.Y.L. is a scholar in the Health Research Board Cancer Scholars programme at UCC and supported by grant PhD/2007/4. We are grateful to Mary McCaffrey and Andrew Lindsay for access to, advice and assistance with confocal microscopy imaging on the Science Foundation Ireland funded (02/IN.1/BO70 to MMC) ZeissLSM 510META.

## References

[1] O. Kerscher, R. Felberbaum, M. Hochstrasser, Modification of proteins by ubiquitin and ubiquitin-like proteins, Annu. Rev. Cell Dev. Biol. 22 (2006) 159–180.
[2] Y. Ye, M. Rape, Building ubiquitin chains: E2 enzymes at work, Nat. Rev. Mol. Cell Biol. 10 (2009) 755–764.
[3] P. de Bie, A. Ciechanover, Ubiquitination of E3 ligases: self-regulation of the ubiquitin system via proteolytic and non-proteolytic mechanisms, Cell Death Differ. 18 (2011) 1393–1402.
[4] S.F. de Almeida, J.V. Fleming, J.E. Azevedo, M. Carmo-Fonseca, M. de Sousa, Stimulation of an unfolded protein response impairs MHC class I expression, J. Immunol. 178 (2007) 3612–3619.
[5] M. Schroder, R.J. Kaufman, The mammalian unfolded protein response, Annu. Rev. Biochem. 74 (2005) 739–789.
[6] M.H. Smith, H.L. Ploegh, J.S. Weissman, Road to ruin: targeting proteins for degradation in the endoplasmic reticulum, Science 334 (2011) 1086–1090.
[7] S. Tiwari, A.M. Weissman, Endoplasmic reticulum (ER)-associated degradation of T cell receptor subunits. Involvement of ER-associated ubiquitin-conjugating enzymes (E2s), J. Biol. Chem. 276 (2001) 16193–16200.
[8] U. Lenk, H. Yu, J. Walter, M.S. Gelman, E. Hartmann, R.R. Kopito, T. Sommer, A role for mammalian Ubc6 homologues in ER-associated protein degradation, J. Cell Sci. 115 (2002) 3007–3014.
[9] R.S. Oh, X. Bai, J.M. Rommens, Human homologs of Ubc6p ubiquitin-conjugating enzyme and phosphorylation of HsUbc6e in response to endoplasmic reticulum stress, J. Biol. Chem. 281 (2006) 21480–21490.
[10] B.W. Kim, A.M. Zavacki, C. Curcio-Morelli, M. Dentice, J.W. Harney, P.R. Larsen, A.C. Bianco, Endoplasmic reticulum-associated degradation of the human type 2 iodothyronine deiodinase (D2) is mediated via an association between mammalian UBC7 and the carboxyl region of D2, Mol. Endocrinol. 17 (2003) 2603–2612.
[11] Y. Imai, M. Soda, H. Inoue, N. Hattori, Y. Mizuno, R. Takahashi, An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin, Cell 105 (2001) 891–902.
[12] Y. Imai, M. Soda, S. Hatakeyama, T. Akagi, T. Hashikawa, K.-I. Nakayama, R. Takahashi, CHIP is associated with Parkin, a gene responsible for familial Parkinson's disease, and enhances its ubiquitin ligase activity, Mol. Cell 10 (2002) 55–67.
[13] C.J. Wu, D.B. Conze, X. Li, S.X. Ying, J.A. Hanover, J.D. Ashwell, TNF-alpha induced c-IAP1/TRAF2 complex translocation to a Ubc6-containing compartment and TRAF2 ubiquitination, EMBO J. 24 (2005) 1886–1898.
[14] J.H. Claessen, B. Mueller, E. Spooner, V.L. Pivorunas, H.L. Ploegh, The transmembrane segment of a tail-anchored protein determines its degradative fate through dislocation from the endoplasmic reticulum, J. Biol. Chem. 285 (2010) 20732–20739.
[15] J.V. Fleming, T.C. Wang, The production of 53–55-kDa isoforms is not required for rat L-histidine decarboxylase activity, J. Biol. Chem. 278 (2003) 686–694.
[16] J.V. Fleming, F. Sanchez-Jimenez, A.A. Moya-Garcia, M.R. Langlois, T.C. Wang, Mapping of catalytically important residues in the rat L-histidine decarboxylase enzyme using bioinformatic and site-directed mutagenesis approaches, Biochem. J. 379 (2004) 253–261.
[17] L. Bhatt, C. Murphy, L.S. O'Driscoll, M. Carmo-Fonseca, M.W. McCaffrey, J.V. Fleming, N-glycosylation is important for the correct intracellular localization of HFE and its ability to decrease cell surface transferrin binding, FEBS J. 277 (2010) 3219–3234.
[18] L.M. Fennell, J.V. Fleming, Differential processing of mammalian L-histidine decarboxylase enzymes, Biochem. Biophys. Res. Commun. 445 (2014) 304–309.
[19] M. Kaneko, S. Yasui, Y. Niinuma, K. Arai, T. Omura, Y. Okuma, Y. Nomura, A different pathway in the endoplasmic reticulum stress-induced expression of human HRD1 and SEL1 genes, FEBS Lett. 581 (2007) 5355–5360.
[20] Y. Iida, T. Fujimori, K. Okawa, K. Nagata, I. Wada, N. Hosokawa, SEL1L protein critically determines the stability of the HRD1–SEL1L endoplasmic reticulum-associated degradation (ERAD) complex to optimize the degradation kinetics of ERAD substrates, J. Biol. Chem. 286 (2011) 16929–16939.
[21] Y. Shen, P. Ballar, A. Apostolou, H. Doong, S. Fang, ER stress differentially regulates the stabilities of ERAD ubiquitin ligases and their substrates, Biochem. Biophys. Res. Commun. 352 (2007) 919–924.
[22] R. Soundararajan, J. Wang, D. Melters, D. Pearce, Glucocorticoid-induced Leucine zipper 1 stimulates the epithelial sodium channel by regulating serum- and glucocorticoid-induced kinase 1 stability and subcellular localization, J. Biol. Chem. 285 (2010) 39905–39913.
[23] F. Vilardi, H. Lorenz, B. Dobberstein, WRB is the receptor for TRC40/Asna1-mediated insertion of tail-anchored proteins into the ER membrane, J. Cell Sci. 124 (2011) 1301–1307.
[24] P. Ballar, A.U. Ors, H. Yang, S. Fang, Differential regulation of CFTRDeltaF508 degradation by ubiquitin ligases gp78 and Hrd1, Int. J. Biochem. Cell Biol. 42 (2010) 167–173.
[25] J. Walter, J. Urban, C. Volkwein, T. Sommer, Sec61p-independent degradation of the tail-anchored ER membrane protein Ubc6p, EMBO J. 20 (2001) 3124–3131.
[26] S.G. Kreft, M. Hochstrasser, An unusual transmembrane helix in the endoplasmic reticulum ubiquitin ligase Doa10 modulates degradation of its cognate E2 enzyme, J. Biol. Chem. 286 (2011) 20163–20174.
[27] T. Ravid, M. Hochstrasser, Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue, Nat. Cell Biol. 9 (2007) 422–427.