Cell Research (2011) 21:555-557.
© 2011 IBCB, SIBS, CAS All rights reserved 1001-0602/11 $ 32.00
www.nature.com/cr

RESEARCH HIGHLIGHT

Waste disposal in the endoplasmic reticulum, ROS production and plant salt stress response

Aldo Ceriotti[1]

[1]*CNR - Consiglio Nazionale delle Ricerche, Istituto di Biologia e Biotecnologia Agraria, via Bassini 15, 20133 Milano, Italy*
Cell Research (2011) **21**:555-557. doi:10.1038/cr.2011.18; published online 1 February 2011

Even under normal conditions, both newly synthesized polypeptides and polypeptides that have already achieved a native conformation can misfold and thus be exposed to the risk of aggregation. Misfolded proteins can be toxic to cells, since they may be prone to spurious interactions or be endowed with undesirable biological activities. The list of possible causes that may lead to the production of a misfolded protein is rather long but includes genetic mutations, error in transcription and translation, and cellular alterations, such as heat shock or oxidative stress. Cells are therefore equipped with efficient systems devoted to the identification, the refolding and, if this fails, the destruction of such aberrant polypeptides. A large fraction of the proteins synthesized in eukaryotic cells is initially targeted to the endoplasmic reticulum (ER). From the ER these proteins then traffic along the secretory pathway to be secreted or delivered to vacuoles or lysosomes, or to the different membranes of the endomembrane system. The ER is therefore equipped with a refined system, called ER protein quality control (ERQC), dedicated to the selective retention and elimination of misfolded polypeptides. Under stress conditions that cause increased accumulation of misfolded proteins, this system may be unable to guarantee ER homeostasis, and the avoidance of the accumulation of such aberrant polypeptides. When this happens, the so called unfolded protein response (UPR) is activated, and this leads to increased synthesis of many components of the ER folding and degradation machineries [1].

While it was initially thought that resident proteases may be responsible for the degradation of proteins that misfold in the ER, it became clear later that structurally defective proteins were retrotranslocated to the cytosol, ubiquitinated and degraded by the proteasome in a process called ER-associated degradation (ERAD). This process includes i) a recognition step, that allows the cell to discriminate unfolded proteins from native ones; ii) a translocation step, where the condemned polypeptide is transported, probably through a proteinaceous channel, to the cytosol; and iii) a degradation step. A variety of soluble and membrane proteins, including glycan processing enzymes, chaperones and oxidoreductases, have been implicated in the processing of structurally corrupt molecules within the ER, but membrane-bound complexes containing a ubiquitin ligase play a central role in the recognition, retrotranslocation and ubiquitination of misfolded polypeptides. In yeast, two ubiquitin ligases mediate the degradation of most ERAD substrates. These are the Doa10 ligase, which mainly takes care of proteins with structural defects in their cytoplasmic parts, and the Hrd1 ligase, which is involved in the degradation of soluble proteins and of membrane proteins bearing defects in their transmembrane or ER luminal domains. The Hrd1 ligase acts in concert with a set of factors to build up a membrane-associated ligase complex (Figure 1).

Several lines of evidence indicate that an intact ERQC machinery is important for plant response to environmental stress [2]. Components of the ER folding machinery have been shown to be involved in biotic [3] and abiotic [4] stress resistance. Similarly, components of the UPR transduction pathway have been shown to be implicated in resistance to salt or heat stress [5-7]. The data presented by Liu *et al.* [8] now show that integral ERAD machinery is required for salt tolerance, thus highlighting the role of one further facet of the ERQC system in abiotic stress resistance.

To study the relationship between ERAD and salt stress, Liu *et al.* have performed a functional characterization of the *Arabidopsis thaliana* homologue of yeast Hrd3, an ERAD substrate recruitment factor that has been shown to be part of the membrane bound Hrd1-containing ligase complex [9] (Figure 1). The *Arabidopsis* genome contains two *HRD3* homologues, *HRD3A* and *HRD3B*, but Liu *et al.* found that the

Correspondence: Aldo Ceriotti
Tel: +39 02 23699444
E-mail: ceriotti@ibba.cnr.it

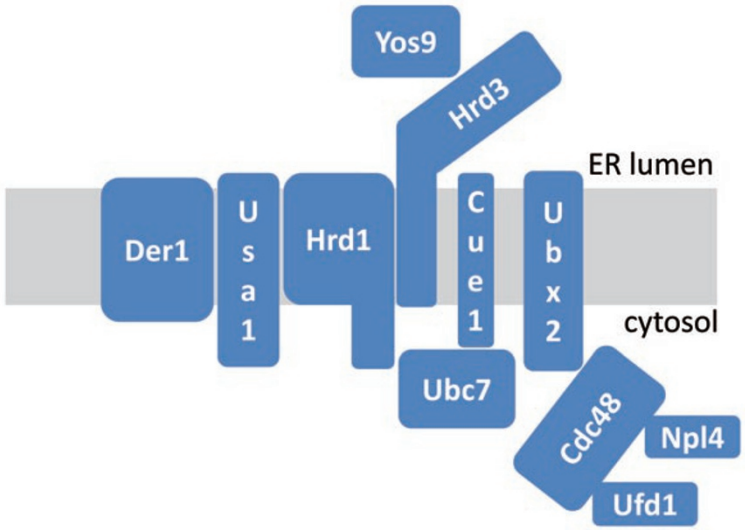

Figure 1 Hrd1-mediated degradation in yeast. Hrd3, in collaboration with the Hrd1 ligase, the ubiquitin conjugating enzyme Ubc7 (that associates with the ER membrane by interacting with Cue1) and the Cdc48/Npl4/Ufd1/Ubx2 complex, can mediate the degradation of most membrane proteins with lesions in their transmembrane or luminal domains. Additional factors (Usa 1 and Der1) participate to the degradation of soluble proteins, while Yos9 acts in concert with Hrd3 in glycoprotein degradation.

latter is probably non-functional, giving rise to an aberrant mRNA in which the second intron has not been removed. A HRD3A-GFP fusion protein localized to the ER and three known ERAD substrates accumulated at much higher level in cells lacking HRD3A. In a parallel study, Su *et al*. have recently identified the same protein as an important component of plant ERAD, and shown that it can complement the ERAD defect of a yeast *Δhrd3* mutant [10]. Crucially, Liu *et al.* found that plants with a defective *HRD3A* gene are more sensitive to salt. While further investigating the effects of *HRD3A* gene disruption, Liu *et al.* also found that *hrd3a* mutants accumulate higher levels of hydrogen peroxide under both normal and salt-stress conditions and are more sensitive to paraquat, an inducer of oxidative stress.

But why should ERAD be relevant to the salt stress response? Salinity has been shown to enhance the production of reactive oxygen species (ROS) [11], toxic molecules that can cause oxidative damage to DNA, lipids and proteins, and that can also lead to protein misfolding. Indeed, Liu *et al.* found that UPR is induced in salt-stressed wild-type plants and that induction is stronger and more prolonged in plants bearing a disrupted *HRD3A* gene. They also found that UPR induction can be blocked by an inhibitor of calcium release from intracellular stores. They propose that sustained UPR would lead to inhibition of plant growth, possibly through an effect on protein synthesis.

In addition, an inefficient ERAD could contribute to ROS-associated injury during salt stress since accumulation of misfolded proteins in the ER has been shown to be associated with ROS production [12] and overexpression of the ER chaperone BiP has been shown to abolish the drought-induced increase in superoxide dismutase activity, an enzyme induced by oxidative stress [4]. Misfolded proteins accumulating in the ER may generate ROS via several possible mechanisms [12]. Repeated cycles of disulfide bond formation and reduction would consume reduced glutathione (diminishing the ability of the cell to eliminate ROS) and would generate hydrogen peroxide, which is produced as a consequence of the transport of electrons from thiol groups in protein substrates to molecular oxygen via the protein disulfide isomerase/Ero1 chain [13]. Further work will be required to define the relative contribution of the ER and of other subcellular compartments to ROS production during salt stress, but the data presented by Liu *et al.* clearly indicate that, in addition to the ER folding and signal transduction machineries, the ERAD pathway should also be considered an important player in plant response to environmental stress.

In yeast, the role of ERAD is not limited to waste disposal, and indeed the HRD3 gene was initially isolated in a screening for mutants that were deficient in the regulated degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, a key enzyme of sterol synthesis [14]. Similarly, in plants, ERAD has been implicated in the post-translational regulation of plant omega-3 fatty-acid desaturases [15]. The ERAD mutant described by Liu *et al.* will therefore be a valuable tool not only to study the turnover of misfolded proteins in plant cells but also to examine the role of ERAD in the regulation of plant metabolism.

References

1 Vitale A, Boston RS. Endoplasmic reticulum quality control and the unfolded protein response: Insights from plants. *Traffic* 2008; **9**:1581-1588.
2 Liu JX, Howell SH. Endoplasmic reticulum protein quality control and its relationship to environmental stress responses in plants. *Plant Cell* **22**:2930-2942.
3 Wang D, Weaver ND, Kesarwani M, Dong XN. Induction of protein secretory pathway is required for systemic acquired resistance. *Science* 2005;

308:1036-1040.

4 Alvim FC, Carolino SM, Cascardo JC, *et al*. Enhanced accumulation of BiP in transgenic plants confers tolerance to water stress. *Plant Physiol* 2001; **126**:1042-1054.

5 Fujita M, Mizukado S, Fujita Y, *et al*. Identification of stress-tolerance-related transcription-factor genes via mini-scale Full-length cDNA Over-eXpressor (FOX) gene hunting system. *Biochem Biophys Res Commun* 2007; **364**:250-257.

6 Gao HB, Brandizzi F, Benning C, Larkin RM. A membrane-tethered transcription factor defines a branch of the heat stress response in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 2008; **105**:16398-16403.

7 Liu JX, Srivastava R, Che P, Howell SH. Salt stress responses in *Arabidopsis* utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. *Plant J* 2007; **51**:897-909.

8 Liu L, Cui F, Li Q, *et al*. The endoplasmic reticulum-associated degradation is necessary for plant salt tolerance. *Cell Res* 2010 Dec 28. doi:10.1038/cr.2010.181

9 Gauss R, Sommer T, Jarosch E. The Hrd1p ligase complex forms a linchpin between ER-lumenal substrate selection and Cdc48p recruitment. *EMBO J* 2006; **25**:1827-1835.

10 Su W, Liu Y, Xia Y, Hong Z, Li J. Conserved endoplasmic reticulum-associated degradation system to eliminate mutated receptor-like kinases in *Arabidopsis*. *Proc Natl Acad Sci USA* 2011; **108**:870-875.

11 Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ* 2010; **33**:453-467.

12 Malhotra JD, Miao H, Zhang K, *et al*. Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc Natl Acad Sci USA* 2008; **105**:18525-18530.

13 Tu BP, Weissman JS. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol* 2004; **164**:341-346.

14 Hampton RY, Gardner RG, Rine J. Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. *Mol Biol Cell* 1996; **7**:2029-2044.

15 O'Quin JB, Bourassa L, Zhang DY, *et al*. Temperature-sensitive post-translational regulation of plant omega-3 fatty-acid desaturases is mediated by the endoplasmic reticulum-associated degradation pathway. *J Biol Chem* 2010; **285**:21781-21796.