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SHORT COMMUNICATION

Defects in endoplasmic reticulum-associated degradation (ERAD) increase selenate sensitivity in Arabidopsis

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ABSTRACT

Stress can impair protein folding in the endoplasmic reticulum (ER). Minimizing the accumulation of misfolded proteins in the ER is achieved by ER-associated degradation (ERAD), which involves the retrograde transport and proteasomal removal of aberrant proteins. Recently, the proteasome has been implicated in a selenium stress response. However, it remains unknown if selenium causes ER stress in plants similar to animals, and if ERAD is associated with optimal selenium tolerance. This deficiency was addressed by monitoring selenate-treated Arabidopsis plants with mutations in HRD1 and SeL1L, participants of ERAD. *hrd1a/hrd1b* and *sel1l* mutants treated with selenate demonstrate decreased tolerance and ER stress, as judged by BiP2 accumulation. The data indicate that optimal plant growth during selenate stress requires ERAD.

Abbreviations: ER, endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; UPR, unfolded protein response; ROS, reactive oxygen species; Se, selenium; WT, wildtype

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Protein quality control is integral to cellular survival, but a variety of abiotic stressors, including heat and salt, can endanger protein structure and consequently lead to misfolding in cells. This is particularly salient in the endoplasmic reticulum (ER) where nascent polypeptides obtain their native confirmation and modifications- including glycosylation- prior to secretion.[1,2] Even though it is estimated that one-third of newly synthesized proteins are misfolded during ambient conditions,[3] ER homeostasis in eukaryotes is maintained by ER-associated degradation (ERAD) of non-native proteins.[4,5] This form of protein quality control in the ER involves the retrograde transport of misfolded proteins into the cytosol, and their subsequent ubiquitination and degradation by the 26S proteasome. Therefore, ERAD prevents misfolded proteins from forming toxic protein aggregates, thereby averting ER stress.

However, ER poise can be disturbed by stressors that overwhelm ERAD's capacity to clear misfolded proteins.[2] For example, tunicamycin and dithiothreitol both result in ER stress by inhibiting glycosylation and reducing disulfide bridges in proteins, respectively. An accumulation of reactive oxygen species (ROS) can also lead to protein misfolding and ER stress.[6,7] In addition to chemicals and abiotic stress, ER stress can be triggered by genetic defects in ERAD, causing an accumulation of unfolded proteins in the ER lumen.[8,9] In each of the cases above, ER stress elicits an unfolded protein response (UPR), which serves to refold proteins by chaperone-mediated processes or degrade irreparable proteins by the ubiquitin-proteasome pathway.[10,11] Additionally, UPR decreases *de novo* protein synthesis to avert further accumulation of misfolded proteins.

Although ERAD is best characterized in yeast, this proteolytic pathway connecting the ER and cytosol also occurs in plants.[4] The recent discovery of HRD1, HRD3A, and OS9 proteins in Arabidopsis indicates that plants and yeast use similarly ERAD machinery, as reviewed.[9] The HRD complex resides on the ER membrane, and is responsible for removing proteins with folding defects exposed to the ER membrane or lumen. (A separate DOA10 complex in yeast removes incorrectly folded proteins facing the cytosol, but is uncharacterized in plants despite the discovery of homologous components).[12] Functionally redundant HRD1A and HRD1B are E3 ubiquitin ligases that assist in protein ubiquitination prior to delivery to the 26S proteasomes.[13] Another component of the HRD complex is HRD3A, the only homolog of yeast HRD3 and mammalian SEL1L.[14] HRD3A and the luminal lectin OS9 are believed to work together to recognize and recruit misfolded proteins to the HRD complex,[7] where aberrant proteins are ultimately retrotranslocated to the proteasome.

The integrity of the HRD complex is associated with stress tolerance in Arabidopsis. Double knockout of HRD1A and HRD1B results in elevated salt sensitivity in *hrd1a/hrd1b* Arabidopsis plants, which was explained by NaCl-induced ROS accumulation that affected the formation of disulfide bridges.[9] Additionally, mutation in HRD3A renders Arabidopsis plants sensitive to salt and tunicamycin. Both stressors induced expression of *BIP1/2*, which encodes an ER chaperone involved in UPR; however, expression was greater in *hrd3a* plants

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compared to wildtype plants, suggesting that ER stress was exacerbated in the mutants.[14]

Whether or not optimal selenium (Se) stress also requires ERAD is unknown. Although plants can accumulate Se, it is not required in higher plants as it is in mammals.[15] Se generates ROS, and its toxicity in plants can partially be attributed to oxidative stress.[16-18] Selenite treatment rapidly induces mitochondrial superoxide in the green algae *Chlamydomonas*.[19] This type of ROS was also observed in selenite-treated *Brassica napus*, and coincided with altered respiration and a mitochondrial stress response.[20] Inorganic Se can be assimilated into Se-cysteine *via* the sulfur assimilatory pathway.[21] The inadvertent replacement of cysteine with selenocysteine in proteins has long been associated with misfolding, and assumed to be another form of Se toxicity.[22,23] Recently, the proteasome has been implicated in the removal of malformed selenoproteins in plants, and supports the misfolded selenoprotein hypothesis. Proteasome inhibition in Se-treated *Stanleya pinnata*[24] and *Chlamydomonas*[19] increases the amount of Se in protein, indicating that functioning proteasomes act to degrade nonspecific selenoproteins. Additionally, Se-cysteine increases proteasome activity in *B. napus*,[25] further suggesting that a cysteine to Se-

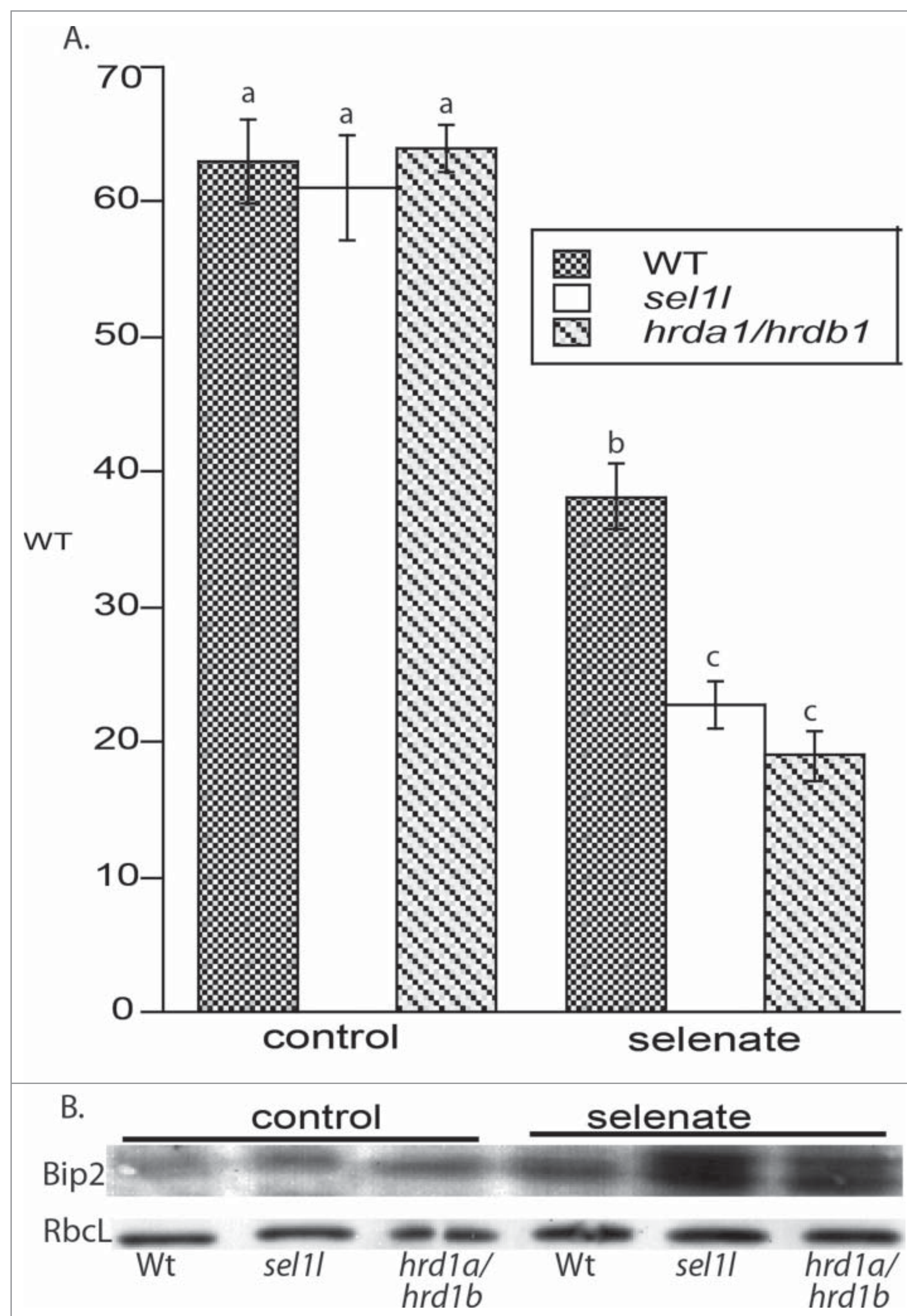

Figure 1. (a) *sel1l* and *hrd1a/hrd1b* plants have decreased tolerance to selenate. Plants were grown on 0.5MS vertical agar plants with or without 40 μM selenate. After 10 d, root lengths were measured. Lowercase letters represent a significant difference between treatments ($p < 0.05$, $n = 30$ replicates). Shown are the mean and SE, and represent another experimental replicate. (b) ERAD mutants accumulate the ER chaperone BiP2 on media with selenate for 10 d. Immunoreactive bands represent 4 other experimental replicates. RbcL- large subunit of RUBISCO

cysteine substitution is toxic. Coinciding with the above data, Arabidopsis mutants with impaired 26S proteasome activity are sensitive to selenate.[24]

The aim of this study was to determine if defects in ERAD would decrease selenate tolerance in Arabidopsis. ERAD mutant plants used in this study were obtained as *hrd1a/hrd1b*[9] and *sel1l*[14] seeds, and compared to wildtype (WT) plants in the same genetic background (ecotype Columbia). Plants were grown in a growth chamber (150 microEinstein, 16 h light/8 h dark cycle, 24°C) on vertical agar plates containing 0.5 strength MS media with or without 40 μM sodium selenate, a concentration known to induce Se stress.[26] To determine selenate toxicity, plants ($n = 30$) were grown with and without selenate. Selenate tolerance was determined by measuring root length in the WT and mutant plants after 10 d. All statistical analyses (ANOVA) were performed using the Kaleida-graph software package (Synergy Software).

There was no difference in plant growth, as determined by measuring root lengths, among the 3 ecotypes on control media as observed earlier.[9,14] Compared to control media, root length in WT plants challenged with selenate decreased by 40%, demonstrating its toxicity (Fig. 1a). Defects in ERAD exacerbated selenate's toxicity. Compared to WT plants treated with selenate, root length of *sel1l* and *hrd1a/hrd1b* decreased by 40% and 50%, respectively. These data suggest that maintenance of ERAD is required for optimal tolerance to selenate.

To determine if Se induces ER stress in WT and ERAD mutants, levels of Bip2 in seedlings were analyzed on a 15% SDS-PAGE gel as previously described.[20] This ER chaperone accumulates in response to stressors that trigger UPR[2,4]. In untreated plants, levels of Bip2 accumulate in ERAD mutants compared to WT. Although Se treatment increases Bip2 levels in WT plants, its accumulation is even more pronounced in the

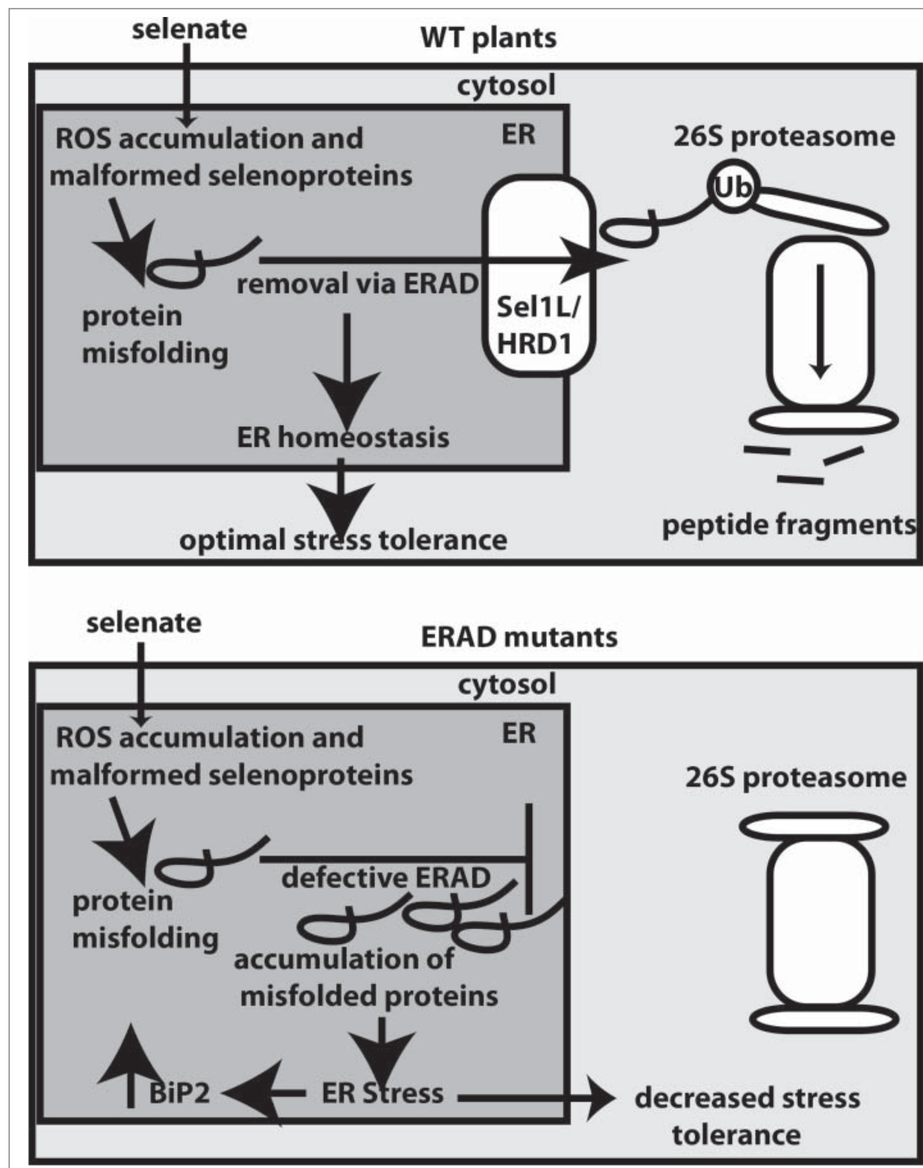

Figure 2. Schematic model depicting Arabidopsis' response to selenate in wildtype (top) and ERAD mutant plants (bottom). In wildtype plants, ERAD removes selenate-induced misfolded proteins from the ER to the cytosol, where the ubiquitinated proteins are degraded by the 26S proteasome. *sel1l* and *hrd1a/hrd1b* plants have defects in ERAD machinery. As a result, misfolded proteins accumulate in the ER, which triggers UPR and increased levels of BiP2. ER stress in the ERAD mutants likely contributes to decreased selenate tolerance. Ub- ubiquitin

ERAD mutants treated with selenate (Fig. 1b); this result coincides with Bip2s accumulation in *hrd3a* plants treated with salt and tunicamycin.[14] The presence of 2 bands in *sel1l* and *hrd1a/hrd1b* plants challenged with selenate probably reflects nonspecific immunoreactivity of the Bip2 antibody against either Bip1 or Bip3 . Levels of the large subunit of RUBICSO (RbcL) were not altered.

Increased levels of BiP2 indicate that selenate induces ER stress during ERAD impairment, which likely explains the elevated sensitivity of the ERAD mutants to Se stress, as depicted in a schematic model (Fig. 2). Selenate sensitivity was also observed in Arabidopsis plants with impaired proteasome activity. Intriguingly, however, selenate did not affect *bip2-1* Arabidopsis plants.[24] This would suggest that Se toxicity does not induce ER stress if intact ERAD machinery can remove damaged proteins from the ER, and deliver aberrant proteins to the proteasome. Computational and manual analysis of transcriptome data[26] further suggest that selenate does not induce expression of common UPR or ER stress-related transcripts.[4]

Because Se toxicity causes both oxidative stress and most likely protein misfolding as a result of a cysteine to Se-cysteine substitution,[18] it is difficult to ascertain why selenate directly caused decreased growth and ER stress in the ERAD mutants. Yeast with defective ERAD machinery display ER stress and sensitivity to canavanine, a structural analog of arginine.[8] Therefore, it is feasible that cysteine's analog Se-cysteine may also cause ER stress in ERAD mutants. In human cells, Se caused an ER stress response, but the accumulation of unfolded proteins in the ER was attributed to Se-induced oxidative stress and altered redox status.[27] In this context, it is worth noting that selenate can deplete the pool of reduced glutathione in Arabidopsis which can perturb redox poise.[17,28]

In conclusion, optimal selenate tolerance in Arabidopsis is dependent upon intactness of the HRD complex, a component of ERAD. Whether or not overexpression of genes participating in ERAD pathway confer Se tolerance is unknown. Because Se is an essential trace element and has purported health benefits in humans, plants with fortified levels of Se and enhanced tolerance have been envisioned to have therapeutic applications.[15] This goal might be achieved in plants with enhanced ERAD efficiency, but this remains to be experimentally tested.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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