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Cloning and Initial Characterization of the *Arabidopsis thaliana* Endoplasmic Reticulum Oxidoreductins

DAVID P. DIXON, MARCEL VAN LITH, ROBERT EDWARDS, and ADAM BENHAM

ABSTRACT

The oxidation and isomerization of disulfide bonds is necessary for the growth of all organisms. In yeast, the oxidative folding of secretory pathway proteins is catalyzed by protein disulfide isomerase (PDI), which requires Ero1p (endoplasmic reticulum oxidoreductin) for its own oxidation. In *Homo sapiens*, two homologues of Ero1p, Ero1-Lα and Ero1-Lβ, have been cloned. Both Ero1-Lα and Ero1-Lβ interact via disulfide bonds with PDI and support the oxidation of immunoglobulin light chains. However, the function of Ero proteins in plants has not yet been analyzed. In this article, we report the cloning of the two Ero1p homologues present in *Arabidopsis thaliana*, demonstrating that one of the cDNAs has a shorter terminal exon than predicted and differs from the annotated sequence found in the genome database. Sequence analysis of the *Arabidopsis* endoplasmic reticulum oxidoreductins (*AERO*s) reveals that both *AERO1* and *AERO2* are more closely related to each other than to either of the human Eros. Both *in vitro* translated AERO proteins are targeted to the endoplasmic reticulum and glycosylated. The ability to use a genetically tractable multicellular organism in combination with biochemical approaches should further our understanding of redox networks and Ero function in both plants and animals. *Antioxid. Redox Signal.* 5, 389–396.

INTRODUCTION

DISULFIDE BOND FORMATION in prokaryotic and eukaryotic proteins is controlled by a series of oxidoreductases in both the bacterial periplasm and eukaryotic endoplasmic reticulum (ER), respectively (6). In prokaryotes, for example, DsbA and DsbB proteins form a functional pair. Here, DsbA oxidizes periplasmic substrates, and DsbB oxidizes DsbA in a quinone-dependent reaction, with oxygen serving as the ultimate electron acceptor under aerobic conditions (3). In the yeast *Saccharomyces cerevisiae*, a similar principle exists in the ER. Disulfides flow from Ero1p (endoplasmic reticulum oxidoreductin) to protein substrates such as carboxypeptidase Y via protein disulfide isomerase (PDI) proteins (8, 16). *In vitro*, Ero1p requires flavin adenine dinucleotide (FAD) as a cofactor to support the oxidation of disulfide-containing substrates such as RNase A (21). Ero1p binds FAD *in vivo* (20), although it is currently not known how FAD is transported into the ER. Whereas O_2 can act as a terminal electron acceptor for Ero1p in living yeast cells, the potential role of other electron acceptors is again unclear (20). It appears that both an N-terminal CxxxxC motif and a C-terminal CxxCxxC motif are necessary for Ero1p function (9). The N-terminal CxxxxC directly oxidizes Pdi1p, whereas the C-terminal CxxCxxC motif reoxidizes the cysteine residues involved in Pdi1p binding. A detailed molecular model for these events awaits the determination of the Ero1p crystal structure.

S. cerevisiae is unusual in that it only has one Ero protein, which possesses a unique C-terminal tail. The tail probably mediates ER localization, although how other Ero proteins are retained in the ER without this extension is unknown (15). It has been demonstrated that the two mammalian Ero1p homologues, Ero1-Lα and Ero1-Lβ, can restore function to a temperature-sensitive yeast Ero1p mutant (5, 14). Ero1-Lα and Ero1-Lβ

Department of Biological and Biomedical Sciences, University of Durham, Durham, U.K.
Drs. Dixon and Van Lith contributed equally to this work.

both form disulfide bonds with PDI and can interact with the thioredoxin domain-containing protein Erp44 (2). However, neither Ero1-Lα nor Ero1-Lβ can be retrieved in a complex with the other mammalian PDI homologues, including Erp57, Erp72, and P5 (4, 11). This suggests that there is some functional specialization within the PDI family in higher organisms. Both Ero1-Lα and Ero1-Lβ enhance the oxidation of immunoglobulin light chains, with C394A and C397A mutations in the CxxCxxC motif ablating this process (11). Yeast also express Erv2p, an ER resident CxxC-containing flavoprotein that may also support disulfide bond formation in the ER (18). In contrast, animals and plants have no known Erv2p homologues resident in the ER, and the relative contribution of Erv2p to disulfide bond formation in *S. cerevisiae* has been disputed (20).

Our knowledge about disulfide bond formation in eukaryotes has advanced tremendously in recent years. We now appreciate that the making and breaking of disulfides is closely controlled and regulated. However, there are many gaps in our knowledge. It is not clear why some organisms require two Ero proteins with different tissue distribution, what substrate specificity these proteins may have, or whether they can perform different functions within the cell. It is also unclear how and why Ero1-Lα and β interact with PDI, but not with other family members (*e.g.,* Erp57, Erp72, and P5). To address the problem of disulfide bond formation in the ER of a multicellular organism, we are studying the function of oxidoreductases in the model plant *Arabidopsis thaliana.* The extensive resources of the *Arabidopsis* genome project provide an excellent opportunity to study the genetics and biochemistry of disulfide bond formation in a multicellular organism. Higher plants make disulfide-bonded secretory proteins in the ER just like yeast and animals. However, plants also have to cope with a variety of environmental extremes, including anoxic conditions (particularly the roots), free radical damage resulting from photosynthesis, and exposure to fluctuating soil chemistry. Like the ER stress response, some of these conditions require changes in secretory protein production and necessitate a coordinated transcriptional response from the stressed cell or organ. Thus, analyzing how protein oxidation in the ER has adapted to deal with different environmental conditions may be particularly informative.

MATERIALS AND METHODS

Cloning procedures

Arabidopsis endoplasmic reticulum oxidoreductin (*AERO*) clones were amplified by PCR from cDNA prepared from *Arabidopsis* root cultures (ecotype Columbia), treated for 24 h with 1% ethanol (7). To obtain cDNAs for AERO expression, the following primers were used: ERO1a (cgcgcgcatatgggaaaaggcgcaatcaaag), ERO1c (cgcgcgctcgagccagaatgagacagctaaatcc), ERO2a (cgcgcgccatggcggagacggacgtcggaag), ERO2c (cgcgcgctcgagacctcattgaacatctagcttc), and ERO2d (gcgcgcctcgaggcttctcttcccagatacagc). Initially, each *AERO* gene was amplified with a pair of primers, designed using the annotated genome sequence. In all cases, an Advantage PCR kit (Clontech) was used for amplification, with 30 cycles of 94°C for 20 s, 55°C for 30 s, 72°C for 120 s. The genome annotation of *AERO2* appeared incorrect at the 3′ end of the coding sequence, so primer ERO2c was designed to amplify from beyond the likely end of the coding sequence. Following purification and digestion with primer-introduced restriction sites, PCR products were cloned into pET-24a (AERO1 products) or pET-24d (AERO2 products) (both from Novagen) and sequenced. Primer ERO2d was then used together with ERO2a to amplify *AERO2* for cloning into pET-24d with a correctly positioned C-terminal his-tag.

Cell lines

The human fibrosarcoma cell line HT1080 was maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 2 m*M* Glutamax (Invitrogen) and 8% fetal calf serum (Sigma).

In vitro *translation using semipermeabilized cells*

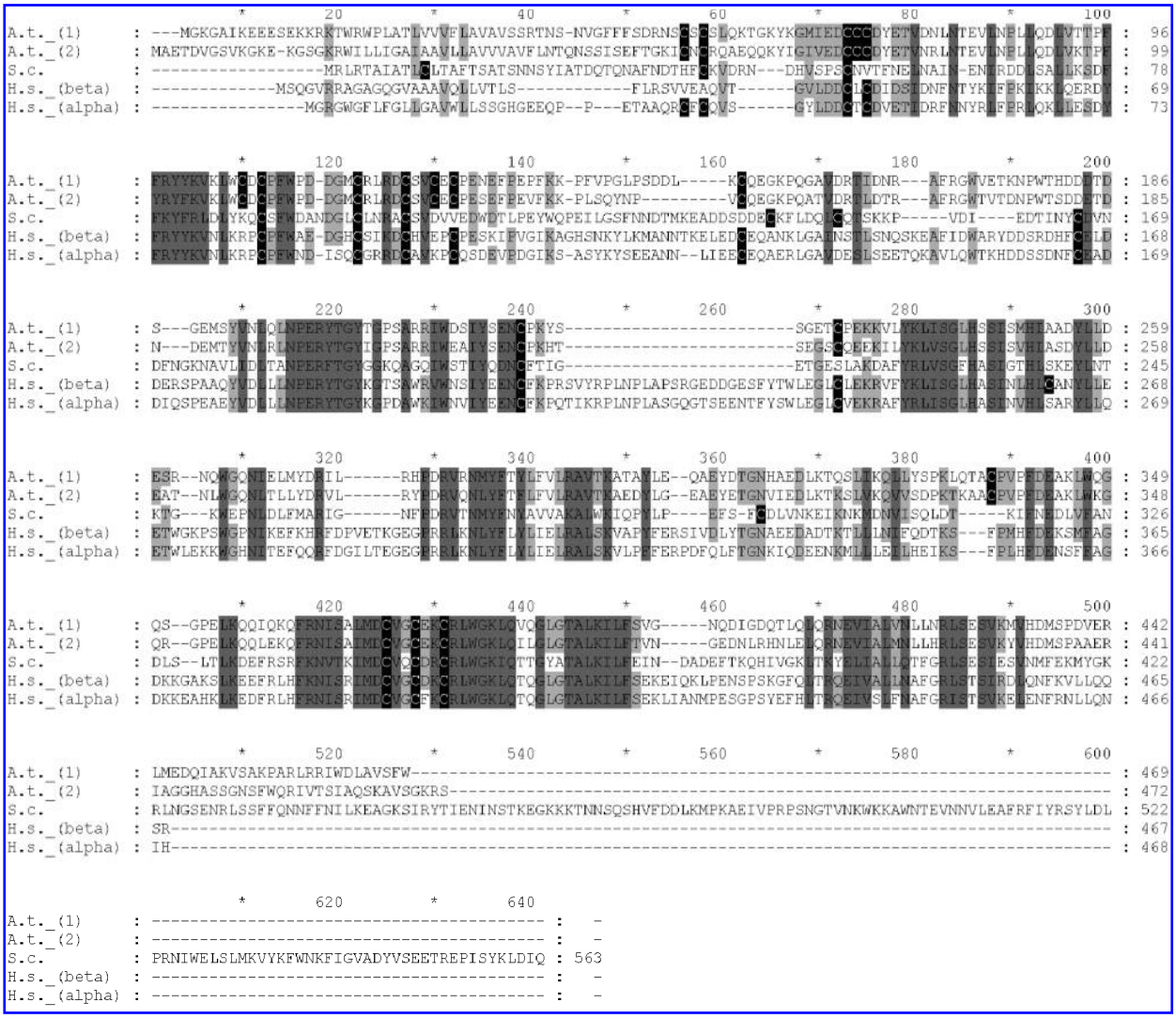
These were performed essentially as described previously (4, 23). The pcDNA3.1-Ero1-Lα construct and the pET-*AERO1* and *2* constructs were first linearized with *Eag1* and *MluI*, respectively. T7 RNA polymerase-driven transcription reactions were performed (Promega). HT1080 cells were selectively permeabilized with 1 μg/ml digitonin in KHM buffer (110 m*M* potassium acetate, 2 m*M* magnesium acetate, 20 m*M* HEPES, pH 7.2) and washed with buffer H (90 m*M* potassium acetate, 50 m*M* HEPES, pH 7.2). After removal of endogenous RNA with micrococcal nuclease, cells were reconstituted with rabbit reticulocyte lysate (Promega) supplemented with 50 m*M* KCl, 0.04 m*M* amino acids, and 10 μCi of *in vitro* ^{35}S-labeling mix (Amersham, U.K.) per 50-μl reaction. Translation was initiated at 30°C upon introduction of the relevant mRNA and continued for 60 min for the pellet samples and 90 min for the concanavalin A (Con A)-Sepharose samples. Translation was stopped by quenching with 20 m*M* *N*-ethylmaleimide on ice to trap free sulfhydryl groups. The SP cells were pelleted by centrifugation (7,500 *g*, 2 min, 4°C) and washed in KHM buffer. Cell pellets were lysed in lysis buffer (20 m*M* MES, 30 m*M* Tris-HCl, 100 m*M* NaCl, pH 7.4, containing 1% Triton X-100 supplemented with 20 m*M* *N*-ethylmaleimide and 10 μg/μl of the protease inhibitors chymostatin, leupeptin, antipain, and pepstatin). Nuclei were spun down at 16,000 *g*, 2 min, 4°C. Lysates were either analyzed directly on 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (after boiling for 3 min in sample buffer containing 50 m*M* dithiothreitol), or were first incubated with 50 μl of a 20% suspension of Con A-Sepharose beads (Amersham) for 1 h at 4°C, to isolate glycoproteins. After the beads were washed twice in wash buffer (300 m*M* NaCl, 10 m*M* Tris-HCl, 0.05% Triton X-100, and 0.05% SDS, pH 8.6), the Con A-Sepharose pellets were analyzed by SDS-PAGE. Gels were dried down and exposed to film for visualization of the radioactive polypeptides.

RESULTS

Cloning of the AERO1 *and* AERO2 *genes*

ERO-like proteins were identified in the *Arabidopsis* genome by BLAST sequence similarity searches (1), using the

(a)



(b)

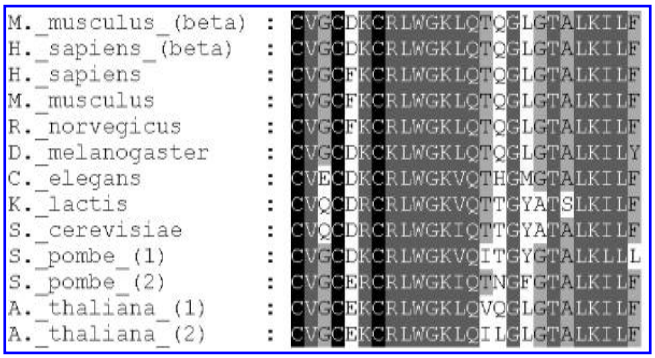


FIG. 1. (**a**) Sequence alignment of AERO1 [A.t. (1)], AERO2 [A.t. (2); both *A. thaliana*], Ero1p [S.c.; *S. cerevisiae*], Ero1-Lα [H.s. (alpha)], and Ero1-Lβ [H.s. (beta); both *H. sapiens*]. (**b**) Comparison of the CxxCxxC motifs from a range of organisms. Cysteine residues are shaded in black, identical residues are shaded in dark gray, and partially conserved amino acids are shaded in light gray. The CxxCxxC motif and surrounding amino acids are well conserved, whereas the N-terminal putative PDI binding site is less well conserved. Note that Ero1p has a unique C-terminal tail.

human Ero1-Lβ protein sequence (14) (GenBank sequence AF252538). These searches identified two genes with significant similarity to the human sequence: gene At1g72280 (encoding a protein that we have named AERO1) and gene At2g38960 (encoding a protein we have named AERO2). The AERO2 polypeptide sequence has been previously aligned with other ERO-like proteins (5). *AERO1* is found on chromosome 1 and *AERO2* is located on chromosome 2. Searches of EST databases showed that ERO-like proteins were also present in other plant species, including tomato, soybean, maize, and barley. At least two *ERO*-like genes are present on chromosome 3 of rice, with reported ESTs. Both *AERO1* and *AERO2* were predicted to encode proteins with putative signal peptides for ER targeting, when analyzed with SignalP (12). Full-length cDNAs were successfully amplified by PCR from material prepared from *Arabidopsis* root cultures, confirming that both genes were expressed. Amplification products were directly cloned into the pET-24 expression vector. Sequencing confirmed that the cDNA encoding *AERO1* was identical to that predicted from the genome sequence. However, the cDNA encoding *AERO2* was different from that predicted (EMBL accession AJ551466). The last intron in the coding sequence was 67 bp longer than expected, resulting in a change of reading frame and a truncated protein sequence that was 45 amino acids shorter with a new AVSGKRS C-terminus.

The AERO1 protein (469 amino acids) and the new, shorter AERO2 protein (472 amino acids) were aligned (Vector NTI) and compared with the full-length sequence of *S. cerevisiae* Ero1p and with the human Ero1-Lα and β proteins (Fig. 1a). The yeast protein is notable in that it has a much longer C-terminal tail not seen in the human or *Arabidopsis* proteins. The signal sequences and N-termini of the proteins showed no significant similarities, and the N-terminal CxxxxC region, which has been implicated in oxidizing PDI, showed a low level of sequence identity. The AERO1 and AERO2 CxxxxC sequences were identical (CRLRDC), but differed significantly from the human and yeast CxxxxC sequences.

In contrast, the alignment showed that the C-terminal CxxCxxC motif and TALK box were strongly conserved between various organisms (Fig. 1b). Human and mouse Ero1-Lα had identical CVGCEKC sequences, whereas human and mouse Ero1-Lβ had identical CVGCDKC sequences. AERO1 and AERO2 both had the same CVGCEKC sequence, and therefore more closely resembled the Ero1-Lβ protein(s), which have a strongly charged CxxCxxC motif. Comparison of all the known CxxCxxC motifs suggested that the "charged" β-like motif is more common, and could be regarded as the ancestral sequence from which the α proteins of human, mouse, and rat subsequently diverged. It will be important to determine the consequences of these amino acid changes in terms of Ero function in both plants and animals.

Phylogeny of the Ero proteins

The aligned sequences of the *Arabidopsis* and human proteins were compared and their sequence identity determined. As previously reported (14), the human Ero1-Lα and β proteins are 65% identical. Our analysis of the AERO1 and AERO2 proteins showed that they are similarly related to each other, being 67% identical. However, both AERO1 and AERO2 were only 31–33% identical to both human Ero1-Lα and Ero1-Lβ. This suggests that the AERO proteins diversified from a single ancestor, possibly as a result of the large-scale gene duplication event that probably occurred in *Arabidopsis* around 65 million years ago.

The aligned Ero sequences from *H. sapiens, M. musculus, R. novegicus, C. elegans, D. melanogaster,* and the yeasts *S. cerevisiae, S. pombe*, and *K. lactis* are displayed as a phylogenic tree (Fig. 2). This analysis verified that AERO1 and AERO2 occupy a distinct evolutionary arm, and that the

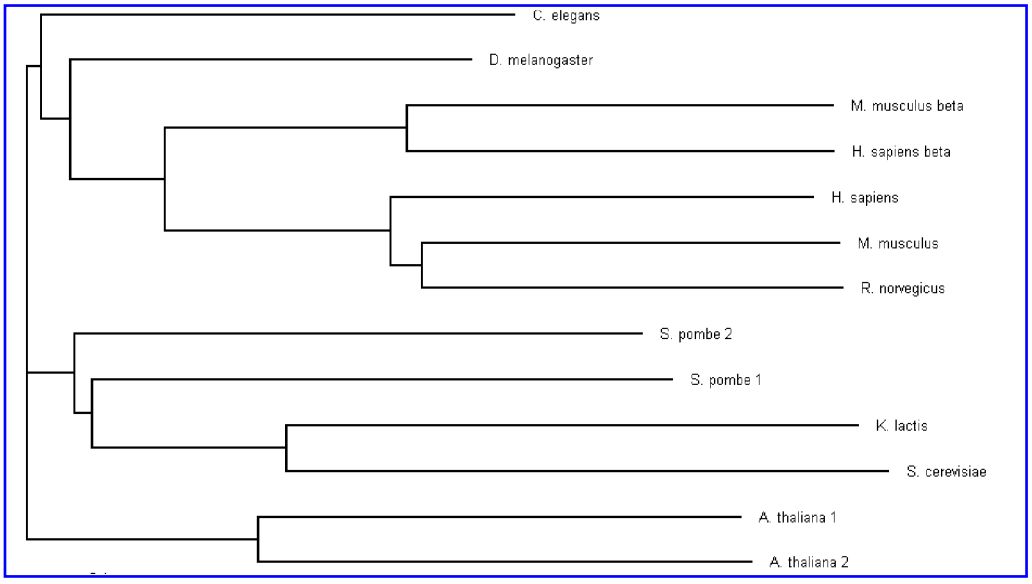


FIG. 2. Phylogenetic tree of the Ero proteins. The AERO proteins are more closely related to each other than to either the α-like or β-like proteins.

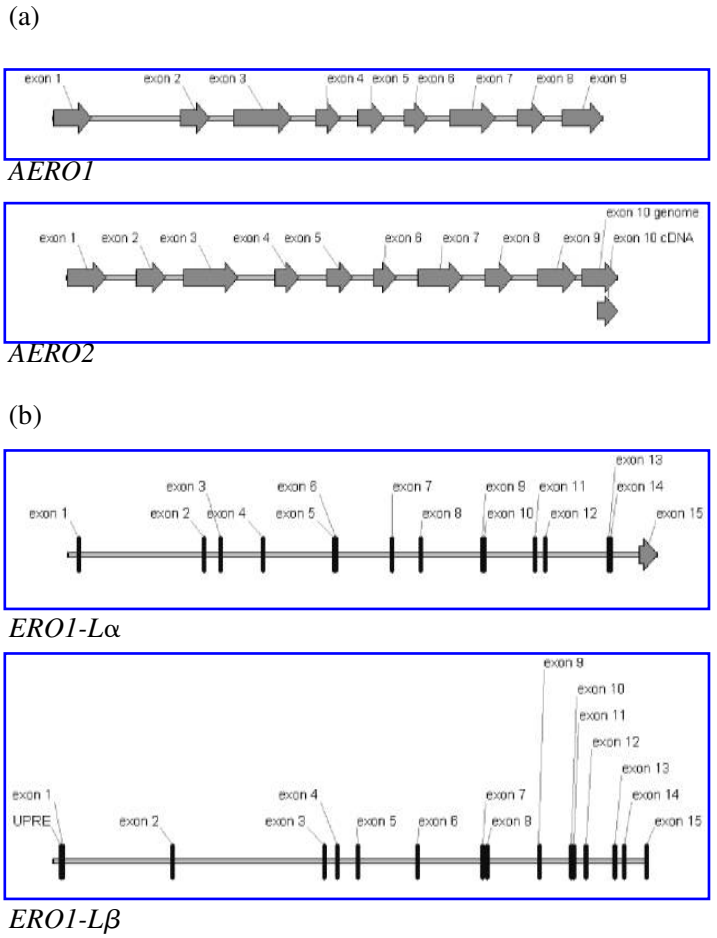


FIG. 3. Exon:intron analysis. (**a**) Exon:intron structure of the *Arabidopsis AERO1* and *AERO2* genes. The alternative final exon 10 of *AERO2* is shown beneath that assigned by the *Arabidopsis* genome annotation. (**b**) Exon:intron structure of the human *ERO1-Lα* and *ERO1-Lβ* genes. The human genes are encoded by four to five more exons than their plant counterparts and occupy more genomic space (not to scale). UPRE, unfolded protein response element.

human, mouse, and rat Ero1-Lαs can be grouped separately from their Ero1-Lβ relatives. Thus far, in the rat, only Ero1-Lα has been archived, and it appears that the nematode and *Drosophila* encode only one Ero protein, despite being multicellular organisms.

Genomic organization of the Arabidopsis and human ERO genes

The organization of the *AERO1*, *AERO2*, *ERO1-Lα*, and *ERO1-Lβ* genes was compared at the genomic level by abstracting information provided by the *Arabidopsis* genome project (www.arabidopsis.org) and the human genome project (www.ensembl.org). The intron:exon arrangements for the *Arabidopsis* and human genes are shown in Fig. 3a and b, respectively. The final exon of *AERO2*, predicted to be longer by the database, is shown above our corrected terminal exon, determined from the cDNA coding sequence (Fig. 3a, lower panel).

The *Arabidopsis* genes occupied less chromosomal space than their human counterparts. Thus, whereas the *AERO1* and *AERO2* genes were accommodated by ~2,500 bp, *ERO1-Lα* and β required 22–26-fold greater nucleotide sequence. Both *ERO1-Lα* and *ERO1-Lβ* contained 15 exons each. AERO1 and AERO2 were encoded by nine and 10 exons, respectively. Both the putative N-terminal PDI-oxidizing CxxxxC motif and the C-terminal CxxCxxC motif were found within intact exons, as expected for a functional domain. The CxxxxC-encoding nucleotide sequences were localized to exon 3 in both plants and humans, whereas the CxxCxxC motif was encoded by exon 8 in *AERO1* and *AERO2*, and by exon 13 in *ERO1-Lα* and *ERO1-Lβ*.

Initial Characterization of the AERO1 and AERO2 proteins

To determine whether or not both *AERO1* and *AERO2* cDNAs could give rise to protein products that were targeted to the ER, the respective mRNAs from the *AERO1* and *AERO2* clones were derived using *in vitro* transcription (Promega). An *in vitro* translation was performed in the presence of semi-permeabilized mammalian HT1080 cells. By using digitonin, the plasma membrane was selectively permeabilized, leaving the ER membranes intact. This technique has been shown to support the correct folding and glycosylation of a number of mammalian proteins (23), including human Ero1-Lα (4), and has the advantage that radiolabeled protein products can be visualized directly without the need for immunoprecipitation.

After translation, the radiolabeled proteins were analyzed by reducing 8% SDS-PAGE. Part of the samples were subjected to a Con A-Sepharose affinity purification step, to isolate glycosylated proteins, before being loaded onto the gel (Fig. 4). AERO2 was resolved as two discrete bands. Compar-

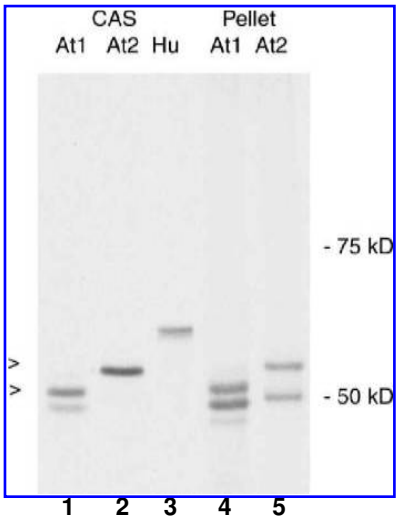


FIG. 4. Translocation and glycosylation of AERO proteins in the mammalian ER. mRNAs corresponding to AERO1 (At1), AERO2 (At2), and Ero1-Lα (Hu) were translated in the presence of [^{35}S]cys/met, using rabbit reticulocyte lysate and digitonin-permeabilized HT1080 cells. Washed cell pellets were directly analyzed by reducing 8% SDS-PAGE (pellet lanes) or after affinity purification using 50 μl of a 20% suspension of Con A-Sepharose beads (CAS lanes). The migration of molecular mass standards is shown. Both AERO proteins are effectively targeted to the mammalian ER and become glycosylated (>). The experiment was repeated three times with similar results (data not shown).

ison with the Con A-Sepharose-purified AERO2 reveals that the upper band was glycosylated AERO2 protein. The lower band corresponded to unglycosylated AERO2. As no intermediates were seen, the mobility shift suggests that all three putative glycosylation sites in AERO2 were used.

In contrast, AERO1 resolved as two strong bands and a fainter lower band. Comparison with the Con A-sepharose-purified AERO1 revealed that both the strong upper band and faint lower band were glycosylated AERO1 protein. AERO1 has one potential (conserved) N-linked glycosylation site. We are currently investigating whether the fainter lower band is the result of a truncated translation product, or a result of proteolysis. Both the AERO1 and AERO2 (major) unglycosylated protein products had the expected molecular mass of ~50 kDa. Expression of recombinant AERO1 in bacteria gave rise to only one protein product of the correct predicted size (data not shown).

This experiment showed that *AERO1* and *AERO2* cDNAs both give rise to glycosylated protein products and that despite having plant signal sequences, these proteins can be effectively targeted to the mammalian ER. Note that it is not uncommon for a fraction of the translated proteins to be ER membrane-associated but not translocated/glycosylated using this system (4). Although we have yet to confirm whether the signal sequences are cleaved by mammalian signal peptidase, the fact that plant ER proteins can be targeted to the mammalian ER by the signal recognition particle (SRP) is of evolutionary and biotechnological significance.

DISCUSSION

In this article, we have taken the first steps toward investigating pathways of ER disulfide bond formation in plants. This is a subject of considerable interest, from the perspective of understanding the biochemistry of disulfide bond formation in a multicellular organism, and from the perspective of biotechnology and crop science. For example, correct disulfide bond formation is essential in the production of functional antibodies in plants ("plantibodies"), which have potential for use as human vaccines (19). It has been demonstrated that the secretion of functional antibodies by tobacco protoplasts requires the ER resident protein BiP (13). However, whether the associated folding of the foreign plantibody immunoglobulin chain requires PDI and Ero homologues is not known. As PDI and Ero are required for oxidation of antibody light chains in human cells (11), it seems likely that this will be the case in plants as well. Surprisingly, oxidized antibody chains have been retrieved from the normally reducing environment of the transgenic plant cell cytosol (17). This illustrates the need to understand, optimize, and control the redox chemistry of recombinant disulfide-bonded proteins if we are to harness the full biotechnological potential of plant culture systems.

Our results demonstrate that *A. thaliana*, like man, has two Ero1p-related oxidoreductases, AERO1 and AERO2 (Figs. 1 and 4). cDNA sequencing has revealed that AERO2 is different from the protein predicted from the database. An inspection of the DNA sequence showed that *AERO2* probably uses an alternative downstream splice site, resulting in a truncated final exon (Fig. 3). However, it will be important to check different *Arabidopsis* tissues, in addition to root cultures, to determine whether alternative splicing can occur in a tissue-specific manner.

There are some interesting sequence similarities and differences between the Ero proteins (Fig. 1). For example, the CxxCxxC motif has been implicated in the recharging of the N-terminal CxxxxC site, which (in *S. cerevisiae* at least) is thought to be involved in PDI oxidation (9). Comparison of human and mouse Ero1-Lα and Ero1-Lβ shows that there is a nonconserved amino acid change within the CxxCxxC site (14). This F for D substitution might influence how readily reoxidation occurs after "charging" of PDI, implying that Ero1-Lα and β are functionally distinct. Alternatively, the function of Ero1-Lα could be essentially indistinguishable from that of Ero1-Lβ, with the overall oxidation capacity being most important. In this case, the ability to up-regulate Ero1-Lβ during ER stress responses would be the crucial feature, rather than any amino acid differences between Ero1-Lα and Ero1-Lβ *per se*.

The alignment of the *Arabidopsis* AERO proteins may shed some light on this question (Fig. 1). AERO1 and AERO2 had identical "β-like" CxxCxxC motifs and identical N-terminal CxxxxC domains. These two proteins are therefore likely to have the same redox capacity, based on amino acid composition of these sequences alone. In addition, analysis of the 5′ untranslated regions of *AERO1* and *AERO2* reveals that *AERO1* has multiple potential unfolded protein response elements and abscisic acid response elements, whereas *AERO2* possesses none of these transcriptional regulatory sites (data not shown). This may indicate that, like mammals, plants have evolved one housekeeping oxidoreductin and one inducible oxidoreductin, and that the ability to control the supply of oxidizing equiva-

lents during ER stress is a critical feature. It also suggests that the abscisic acid-mediated response (*e.g.,* to water stress) and the ER stress response (to accumulated unfolded proteins in the ER) could be coordinated at the transcriptional level, at least in part, by invoking *AERO1* expression. We are now undertaking experiments to determine whether AERO1 is genuinely induced by ER stress and, if so, to identify which transcription factors are involved in this process.

Our experiments using semipermeabilized HT1080 cells show that it is possible to translate plant ER proteins in animal systems (Fig. 4). The SRP, which helps target signal peptides to the ER (in eukaryotes) has been extensively studied in prokaryotes and mammalian cells, and the key components are well conserved (10). It is known that within an organism, SRP can recognize a diverse range of signal peptides, so long as they exhibit a minimum degree of hydrophobicity and can adopt an α helical conformation (22). However, less is known about the function of plant SRPs, and our studies illustrate that signal peptide recognition is flexible between, as well as within, species. Although human Ero1-Lα and Ero1-Lβ both form disulfide-bonded complexes with PDI (4, 11), we have not yet been able to trap either AERO1 or AERO2 with human PDI (data not shown). A failure of AEROs to interact with human PDI is most probably explained by the diversification of the N-terminal CxxxxC region, implicated in Ero-PDI oxidation. This is supported by our observation that the plant and animal N-termini of Ero are poorly conserved, compared with the CxxCxxC motif (Fig. 1). Both AERO1 and AERO2 have additional upstream and downstream cysteine residues in this region that could alter the structure and disulfide bonding characteristics of the respective proteins. PDI binding experiments using a mutant Ero1-Lα in which the human CxxxxC motif is replaced with the plant motif may be informative in this respect. It is worth noting that the N-terminal cysteine-rich domain and the C-terminal CxxCxxC motif of AERO1 are 100% identical to the corresponding regions in the full-length rice homologue. There appears to be no comparable, independent divergence in plants to the "α-like" CVGCFKC sequence that is seen in mammals. This event seems to have occurred only once, relatively late in evolution, and it will be interesting to determine the reason for this difference.

The localization and tissue distribution of endogenous AERO1 and AERO2 in *Arabidoposis* is currently unknown. *Arabidopsis* encodes around nine putative PDI-like proteins (but no obvious Erp44 homologue), and we do not yet know which PDIs act as acceptors of AERO oxidizing equivalents. We are currently investigating these questions as we strive to discover how oxidative protein folding is catalyzed and regulated in plants. By studying a multicellular organism that has to deal with the same ER stresses as animals, but also has to regulate the oxidative challenge of photosynthesis, we hope to make progress toward understanding how separate oxidative processes are integrated and controlled.

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ABBREVIATIONS

AERO, *Arabidopsis thaliana* endoplasmic reticulum oxidoreductin; Con A, concanavalin A; ER, endoplasmic reticulum; ERO, endoplasmic reticulum oxidoreductin; FAD, flavin adenine dinucleotide; PDI, protein disulfide isomerase; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SRP, signal recognition particle.

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Address reprint requests to:
Dr. Adam Benham
Department of Biological and Biomedical Sciences
South Road
University of Durham
Durham
DH1 3LE, U.K.

E-mail: adam.benham@durham.ac.uk

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