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ORIGINAL PAPER

Endoplasmic reticulum stress activates the expression of a sub-group of protein disulfide isomerase genes and AtbZIP60 modulates the response in *Arabidopsis thaliana*

Dong-Ping Lu · David A. Christopher

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Abstract Proteins entering the secretory pathway of eukaryotic cells are folded into their native structures in the endoplasmic reticulum (ER). Disruption of protein folding causes ER stress and activates signaling cascades, designated the unfolded protein response (UPR), that restore folding capacity. In mammals and yeast, the protein disulfide isomerases (PDIs) are key protein folding catalysts activated during UPR. However, little is known about the response of *PDI* genes to UPR in plants. In *Arabidopsis thaliana*, we identified 12 *PDI* genes that differed in polypeptide length, presence of signal peptide and ER retention signal, and the number and positions of thioredoxin and transmembrane domains. *AtPDI* gene expression was investigated in different tissues, in response to chemically induced UPR, and in null mutants of UPR signaling mediators (AtIRE1-2 and AtbZIP60). The expression of six *AtPDI* gene*s* was significantly up-regulated by UPR and sharply attenuated by the transcription inhibitor, actinomycin D, indicating UPR induced *AtPDI* gene transcription. *AtPDI* and *BIP2* (Binding protein) gene expression was not affected in the *Atire1-2* mutant exposed to UPR, however, the expression of four *AtPDI* genes was decreased in the *Atbzip60* mutant. We proposed that additional UPR signaling factors complement AtbZIP60 in the activation of *AtPDI* gene expression during ER stress in plants.

Keywords Thioredoxin · Disulfide modification · Secretory · bZIP60 transcription-factor · Unfolded protein response

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D.-P. Lu (✉) · D. A. Christopher
Department of Molecular Biosciences and Bioengineering,
University of Hawaii, 1955 East-West Rd.,
Honolulu, HI 96822, USA
e-mail: dchr@hawaii.edu

Introduction

Proteins entering the secretory pathway of eukaryotic cells are modified and folded into their native structures in the endoplasmic reticulum (ER). Protein folding is an active process assisted by catalysts and chaperones, some of which are immunoglobulin heavy chain-lumen binding protein (BiP), calreticulin, calnexin and protein disulfide isomerase (PDI) (Gething 1999; Caramelo et al. 2004; Wilkinson and Gilbert 2004). This quality control system ensures that only properly folded proteins exit the ER (Trombetta and Parodi 2003). If protein modification and folding are disturbed, the accumulation of unfolded proteins causes ER stress. To recover, the cell has evolved an elegant mechanism, designated the unfolded protein response (UPR), which returns the protein folding process to its normal state (Harding et al. 2002).

During UPR, the state of protein folding in the ER is sensed and the expression of genes for protein folding enzymes and chaperones is stimulated, while secretory protein gene expression diminishes (Mori et al. 2000; Ron 2002; Martinez and Chrispeels, 2003; Trombetta and Parodi 2003). The transmembrane ER protein kinase, PERK, attenuates secretory protein mRNA translation (Harding et al. 2000; Harding et al. 2002). Finally, misfolded proteins are removed by conjugation to ubiquitin for proteasomal degradation (Travers et al. 2000; Bays et al.

2001; Kostova and Wolf 2003; Kamauchi et al. 2005). Together, these processes increase the folding capability and decrease the pool of nascent proteins that need to be folded in the ER (Mori et al. 2000).

The UPR signal transduction pathway has been extensively characterized in mammals and yeast. Some pathway homologs, such as BiP, IRE1, ATF6, calreticulin, and PDI, have been identified in plants. Excess unfolded proteins in the ER bind to BiP, which is released from the hybrid lumen sensor, protein kinase-ribonuclease, IRE1, leading to its dimerization and activation (Bertolotti et al. 2000; Koizumi et al. 2001; Noh et al. 2002; Okushima et al. 2002). In animals, IRE1 splices the X-Box binding protein (XBP1) mRNA (Yoshida et al. 2001), allowing synthesis of XBP1, a bZIP transcription factor (Lee et al. 2003; Kanemoto et al. 2005). In mammals, the release of BiP can also activate the ATF6 transcription factor, which mediates activation of UPR regulated genes (Lee et al. 2002). An ATF6-like activating transcription factor, AtbZIP60, has recently been found in plants (Iwata and Koizumi 2005). However, the XBP1 transcription factor and the translational regulator, PERK (Harding et al. 2000), have not been found in *Arabidopsis*, raising questions about the nature of the components and the role of translation in the UPR signaling pathway in plants (Kamauchi et al. 2005). Recently, it was found that salt stress signaling utilizes components of ER stress signaling (Liu et al. 2007a).

A key downstream component of UPR, which is present in all eukaryotes with sequenced genomes, is the highly abundant ER oxidoreductase, PDI (EC 5.3.4.1), PDI catalyzes thiol-disulfide interchange reactions that form, break, and isomerize disulfide bonds in substrate proteins (Noiva 1999). The classical PDI has four thioredoxin-like domains, *a–b–b′–a′* (Darby et al. 1996; Gruber et al. 2006). The *a* and *a'* are the thioredoxin catalytic domains that contain the active site, PW**C**GH**C**K (Edman et al. 1985). The *b* and *b′* are two homologous domains with folded structures similar to thioredoxin, but without the active site or thioredoxin sequence homology (Lucero and Kaminer 1999). Recently, a genome wide analysis of gene expression in response to UPR in *Arabidopsis* found some *PDI* genes to be upregulated (Kamauchi et al. 2005). However, despite the importance of PDIs in UPR, the systematic analysis of *PDI* gene expression in response to ER stress has not, to date, been conducted in plants.

The study of PDIs in plants requires a clear definition of the features that specifically constitute a PDI. In this report, we used stringent bioinformatic criteria to define and characterize a 12-member *Arabidopsis PDI* gene family (*AtPDI)*. We then analyzed the expression of *AtPDI* genes in different tissues, in response to chemically induced ER stress and in UPR-signaling mutants. *BiP2* gene expression served as an internal control verifying UPR activation. We used three chemicals, dithiothreitol (DTT), tunicamycin (Tm) and beta-mercaptoethanol (β-Me), which are known to induce the accumulation of unfolded proteins and activate ER stress and UPR (Martinez and Chrispeels 2003; Noh et al. 2003; Kamauchi et al. 2005). The reducing agents, DTT (dithiol) and β-Me (monothiol), break disulfide bonds and inhibit their formation (Jämsä et al. 1994; Back et al. 2005). Tm inhibits asparagine N-linked glycosylation (Takatsuki et al. 1971), which is a prerequisite for glycoprotein folding (Dorner et al. 1990). In *Arabidopsis*, DTT and Tm induce the expression of genes for protein folding enzymes and decrease secretory protein gene expression (Martinez and Chrispeels 2003). Here we found that the expression of a sub-group of six *AtPDI* genes was increased in response to ER stress. The transcription inhibitor, actinomycin D, significantly attenuated this response. We identified null mutants, *Atire1-2* and *Atbzip60*, and investigated *AtPDI* gene expression in these mutants exposed to ER stress. We found that AtbZIP60 modulates the up-regulation of a subgroup of *AtPDI* genes in response to UPR. This work paves the way for extended studies of a gene family of fundamental importance to all eukaryotes, Besides providing insight into the UPR in plants, this work also highlights evolutionary differences in the UPR signaling between plants, yeast and mammals.

Materials and methods

Plant material and growth conditions

For chemical treatments, wild type *Arabidopsis thaliana* L. Heynh (ecotype Columbia) and T-DNA insertion mutant seeds were surface-sterilized, and placed in the dark for 2 days at 4°C. They were germinated in 50 mL of liquid Murashige and Skoog medium, containing 3% Suc (w/v), in a 16-h-light/8-h-dark cycle at 23°C with constant shaking (50 rpm) for 7 or 16 days. *Arabidopsis* seeds were also grown in soil in the same environmental conditions as liquid-cultured plants for 16 days for T-DNA insertion genotyping and 2 months for tissue dissection.

Chemical treatments

One-week-old liquid cultured *Arabidopsis* plantlets were treated with 5 μg/ml Tm or 20 mM β-Me for 0, 2, or 5 h as described by Martinez and Chrispeels (2003). Sixteen-day-old liquid-cultured plantlets were treated with 10 mM DTT for 0, 2, or 5 h as described by Martinez and Chrispeels (2003). In some experiments, 9-day-old liquid cultured plants were pre-treated with actinomycin D at final concentration of 25 μg/ml for 3 h. Then the plants were subjected

to 5 μg/ml Tm for 0, 2 and 5 h. The control for Tm treatment was a final concentration of 1% DMSO, which was the solvent for Tm. After the chemical treatments, plants were harvested and total RNA isolated.

RNA isolation and RT-PCR

Total cell RNA was isolated as described (Hoffer and Christopher 1997) from whole seedlings or from roots (R), stems (S), rosette leaves (RL), cauline leaves (CL), and flowers (F) of 2-month-old *Arabidopsis* plants grown on soil. For reverse transcription PCR (RT-PCR), RNA was treated with RNase-free DNase (Promega, Madison, WI, USA) and used to produce the first-strand cDNA with M-MLV reverse transcriptase and oligo dT (Promega). 50 ng of total RNA was used to amplify the transcripts of each AtPDI, actin, calreticulin, and AtbZIP60 gene by RT-PCR for the indicated number of cycles in each figure. The cDNA mixtures used for PCR were the Bio-X-Act Short Mix (Bioline Inc., Taunton, MA, USA). The gene-specific primers used in the study corresponded to exon–exon junctions to avoid amplification of genomic DNA, and they are listed in Table 1. The PCR products were sequenced to confirm their identity.

RNA gel blot analysis

RNA was separated and transferred to nylon membrane as described (Chun et al. 2001). The ^{32}P-labeled cDNA probe was made using a DECAprimer II labeling kit (Ambion, TX, USA). The cDNA inserts (*Atpdi2* to *Atpdi11)* were generously provided by Dr. Danon (Weizmann Institute of Science). Hybridization and radioactive signal detection were performed as described (Christopher and Mullet 1994).

Genotyping of T-DNA insertion mutants

The T-DNA insertion in each mutant was detected by PCR using a gene specific primer and a T-DNA left border primer, LBa1. The accurate insertion positions were determined by sequencing each PCR product using the LBa1 primer. The genotypes for T-DNA insertion were determined by PCR using two gene-specific primers.

Computer analysis of sequences

Clustal W (http://align.genome.jp/) was used for the multiple sequence alignments and for pairwise analysis of the phylogenetic relationship of the *Arabidopsis* PDI proteins. The pairwise analysis used a gap penalty of 10 and a gap extension penalty of 0.05 (http://align.genome.jp/). Based on the multiple sequence alignment of the 12 *AtPDI*s, an unrooted phylogenetic tree was constructed using "Neighbour Joining" algorithm (Saitou and Nei 1997) wthin the Phylo-win program (Galtier et al. 1996). A bootstrap analysis with 1,000 replicates was performed and the bootstrap values are shown in Fig. 1a. Signal P3.0 (http://www.cbs.dtu.dk/services/SignalP/) was used to predict the presence of signal peptides. TMHMM ver. 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/) was used to determine transmembrane domains. The subcellular localizations of AtPDIs were predicted by using TargetP1.1 (http://www.cbs.dtu.dk/services/TargetP/) and pSORT (http://wolfpsort.org/).

Results

Sequence analysis of AtPDIs

Using the classical human PDI (HsPDI) as a standard (Darby et al. 1996; Tasanen et al. 1998), we defined 12 members of the PDI family, ranging in size from 361 to 597 amino acids (Fig. 1a, b; Table 2). Their sequences were aligned with HsPDI (Supplemental Fig. 1). Ten PDIs (AtPDI1, AtPDI2, AtPDI3, AtPDI4, AtPDI5, AtPDI6, AtPDI8, AtPDI9, AtPDI10, and AtPDI11) and HsPDI were anchored at a conserved thioredoxin domain proximal to the N-termini, designated classical domain a (Fig. 1b). The second thioredoxin domains of HsPDI, AtPDI1, AtPDI2, AtPDI3, AtPDI4, AtPDI5 and AtPDI6 were collinear and designated classical domain a′ (Fig. 1b). As with HsPDI, the fold domains b and b′ are located between domains a and a′ in these six plant sequences (Fig. 1b). Thus, HsPDI, AtPDI1, AtPDI2, AtPDI3, AtPDI4, AtPDI5 and AtPDI6 share the standard PDI domain architecture: a–b–b′–a′. The second thioredoxin domains of AtPDI9, AtPDI10, AtPDI11, and the single corresponding domains of AtPDI7 and AtPDI12, aligned together. These thioredoxin sites are closer to the N-termini in AtPDI9, AtPDI10, AtPDI11 than in AtPDI1, AtPDI2, AtPDI3, AtPDI4, AtPDI5, and AtPDI6. No homologous b or b′ fold domain could be found in AtPDI9, AtPDI10, and AtPDI11. Recently, the orthologs of *Arabidopsis* AtPDI11 in soybean, GmPDIS-1 and GmPDIS-2, were identified to have the domain organization of a–a′-ERp29c, without b or b' domains (Wadahama et al. 2007).

Ten PDIs are predicted to have a signal peptide at the N-termini (scores 67–100% probability, Table 2; Fig. 1b), which indicates that they are translocated over the ER membrane into the lumen and possibly further into the secretory pathway (Bendtsen et al. 2004). However, AtPDI1, AtPDI2, AtPDI4, and AtPDI10 also have secondary predictions for the chloroplast (Table 2). AtPDI7 and AtPDI8 lack signal peptides altogether and have scores

Table 1 The gene-specific primers used this study

Primer name	Sequence (5′ to 3′) or reference source
AtPDI1 forward	ACCGAGTTCGTCGGCAACAATAGC
AtPDI1 reverse	CCACAATGACCTTAACATCACCATCG
AtPDI2 forward	TGCCACGGAGCTTAAGGAAGATGG
AtPDI2 reverse	ACACCATGGTGCGTAGACCTCG
AtPDI3 forward	GATTCCAATGTCGAATCAAACGAACCC
AtPDI3 reverse	GTTTGGTGATGGATCTGACTCGAGC
AtPDI4 forward	TGGTGTGCGAGGAGTGCTGAGC
AtPDI4 reverse	CTAGGAGATGGATCCGACTCAAGC
AtPDI5 forward	Noh et al. (2003)
AtPDI5 reverse	Noh et al. (2003)
AtPDI6 forward	TCACCGAAACCATCTCCAAGCACG
AtPDI6 reverse	TCCGTCCTGGAAGTCCTTGAACC
AtPDI7 forward	TGACAGAGGCGTCTCTGTCTGG
AtPDI7 reverse	ATGGTCGTGGTGTGCATTGTCATCC
AtPDI8 forward	TATGCTCCTTGGTGTGGTCACTGC
AtPDI8 reverse	ACCTGATCATACTCTTCATCTCCATCC
AtPDI9 forward	ATGTATAAATCACCATTAACGTTGCTCACAC
AtPDI9 reverse	TCACAACTCATCCTTAGAACCAACAGC
AtPDI10 forward	CTGTTGTTTGCCTTGTTCGATCGC
AtPDI10 reverse	ATAGCTGATGCAGATCTAGCACCC
AtPDI10 forward	TTGCGTTACTCGCGTTGCTTCTGG
*AtPDI11*reverse	AGCTTTCCATACCTCGTGGTGGAG
AtPDI10 forward	ACGGAGGCATCTCTATCAGGC
AtPDI12 reverse	ACTGTCTGTGTCCCCGATCTCC
Actin2 forward	TTGCAGGAGATGATGCTCCCAGG
Actin2 reverse	CATTCCCACAAACGAGGGCTGG
AtbZIP60, LP1	TGGCGGAGGAATTTGGAAGCATAG
AtbZIP60, LP2	AGTCTTCACCGGATTCATGGATCG
AtbZIP60, RP	AGACTCCTGCTTCGACATCATGG
BiP2 forward	Noh et al. (2003)
BiP2 reverse	Noh et al. (2003)
Calreticulin forward	Noh et al. (2003)
Calreticulin reverse	Noh et al. (2003)
AtIRE1-1 forward	AAACCAGTTCCCGCTGTCTTA
AtIRE1-1 reverse	TCCCTTGCCATTCTCAAAGAT
AtIRE1-2 forward	ATTGCAAAGGGAAGTAACGGA
AtIRE1-2 reverse	AGATCATCACCAAAGGGATGC

(TargetP, pSORT) for entering the "other" subcellular locations or mitochondria (Table 2). AtPDI8 has a high score for the plasma membrane. The C-terminal ER retention signal, KDEL, is conserved in AtPDI1, AtPDI2, AtPDI3, AtPDI4, AtPDI5, AtPDI6, AtPDI9, and is partially conserved in AtPDI10 (KDDL), further suggesting that these are ER proteins. AtPDI8 has a KKXX-like tetrapeptide, KKED, at its C-terminus, which serves as an ER retention signal for a membrane protein (Vincent et al. 1998). Three of the proteins are predicted to have transmembrane domains (TMD). AtPDI8 has a TMD in the C-terminus, whereas AtPDI7 and AtPDI12 have them in both the N-and C-termini.

Pairwise comparisons were used to identify five pairs of closely related PDIs (Fig. 1a). The pairs are AtPDI1 and AtPDI2 (54% identity), AtPDI3 and AtPDI4 (76% identity), AtPDI5 and AtPDI6 (79% identity), AtPDI7 and AtPDI12 (73% identity) and AtPDI9 and AtPDI10 (76% identity) (Fig. 1). This suggests that the genes encoding the each pair of proteins may have arisen from a recent duplication event. The 12 PDIs fall into three divergent subgroups (Fig. 1a). AtPDI9 and AtPDI10, with AtPDI11 form the

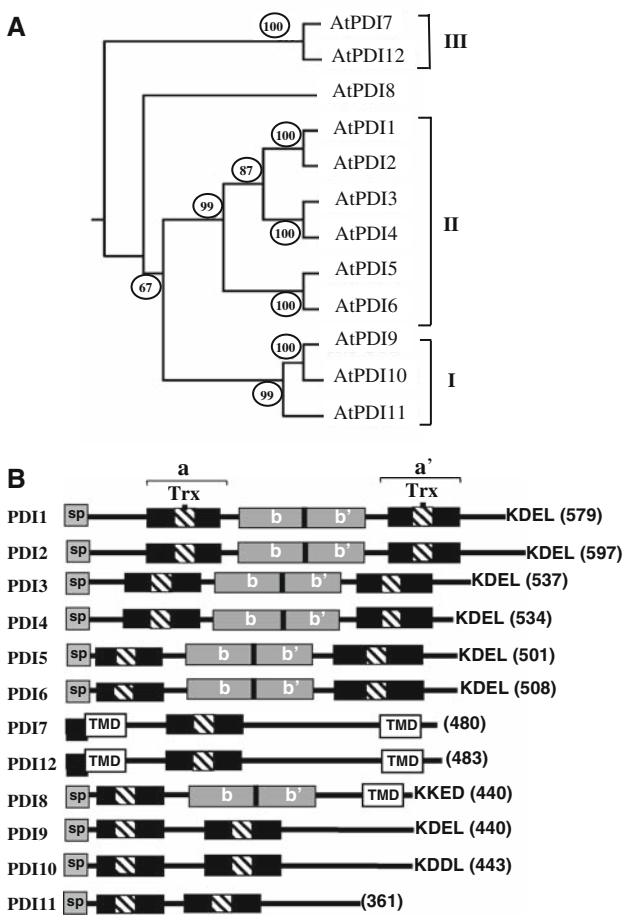

Fig. 1 The phylogenetic relationship and primary structure of 12 AtPDIs. **a** The phylogenetic and pairwise analyses of predicted PDI gene products are shown resulting in three sub-groups. The bootstrap values are circled. **b** The 12 AtPDIs were grouped based on sequence and domain similarity. The predicted number of amino acids encoded by each cDNA is shown in parenthesis at the C-terminus. *SP* signal peptide, *Trx* thioredoxin catalytic domain, *TMD* transmembrane domain; *a* and *a′*, full thioredoxin domains with catalytic site and fold region; *b* and *b′*, thioredoxin fold domains lacking catalytic site

subgroup I, sharing the similar domain organization, but AtPDI9 and AtPDI11, AtPDI10 and AtPDI11 share overall low identity. Within subgroup II, the three PDI pairs all have the standard PDI domain structure a–b–b′–a′. AtPDI8 only has one thioredoxin domain and the standard b and b′ domains, and resides outside all three groups (Fig. 1a). The subgroup III consists of the PDI pair (AtPDI7 and AtPDI12), which have a single thioredoxin domain and two common TMDs. A gene for a thirteenth protein (At1g07960) containing a thioredoxin domain at the same position as AtPDI7 and AtPDI12 was found, but it was completely truncated at the c-terminus. This sequence was considered too divergent from the PDI family to be included, although it was a member of the thioredoxin superfamily.

The relative abundance of the *AtPDI* transcripts in seedlings and in different mature tissues

The relative transcript abundance of the 12 *AtPDI* genes, relative to actin mRNA as a reference control, was examined using RT-PCR of total cell RNA from whole 7-day-old seedlings (Fig. 2a). *AtPDI11*, *AtPDI5, AtPDI9*, *AtPDI2,* and *AtPDI6* have moderately high transcript abundance (Fig. 2a), whereas the abundance of *AtPDI3*, *AtPDI4, AtPDI12*, and *AtPDI7* mRNA was lower, with *AtPDI3* being the lowest. BLAST searches for each *AtPDI* gene in the NCBI *Arabidopsis* EST database confirmed these observations (Fig. 2b). The numbers of ESTs for *AtPDI11*, *AtPDI5, AtPDI2,* and *AtPDI6* were high, with 100–101 ESTs per gene. *AtPDI9* has 63 ESTs, while *AtPDI3*, *AtPDI4* and *AtPDI12* had lower numbers (Fig. 2b) with *AtPDI3* being the lowest (5 ESTs). We also performed RT-PCR analysis on total cell RNA from roots, stems, rosette and cauline leaves, and flowers of a mature plant (Fig. 2c). *AtPDI* genes were expressed in all organs, with the highest expression in flowers. This pattern is similar to that of the ER chaperon gene for calreticulin. In non-floral tissues, the *AtPDI* transcript levels did not differ notably.

The expression of *AtPDI* genes in response to ER stress

To study the expression of *AtPDI* genes in response to ER stress, we treated seedlings separately with three chemical inducers of ER stress: dithiothreitol (DTT), beta-mercaptoethanol (β-Me), and tunicamycin (Tm). The mRNA levels for the *BiP2* gene, which is a known internal molecular marker of ER stress, and the *AtbZIP60* gene, which is a novel transcription factor believed to be involved in ER stress, are induced by the chemical treatments (Fig. 3a). Similarly, the expression of *AtPDI1*, *AtPDI5*, *AtPDI6*, *AtPDI9*, *AtPDI10*, *and AtPDI11* genes are increased in plants treated with the three chemicals (Fig. 3a). Because DTT (β-Me) and Tm induce ER stress by different mechanisms, the increases in mRNA abundance of *AtPDI* genes were very likely to be due to ER stress rather than nonspecific effects of the chemicals. In contrast, these reagents either slightly repress or have minimal effects on the expression of *AtPDI2*, *AtPDI7*, and *AtPDI8*. Due to low abundance, the mRNAs of *AtPDI3*, *AtPDI4* and *AtPDI12* were barely detected by RNA gel-blot analysis. Thus, RT-PCR was performed to determine their expression (Fig. 3b). Their transcript levels decreased, or had minimal changes, upon chemical treatments.

ER stress increases *AtPDI* gene transcription

To study whether the induction of the *AtPDI* genes was regulated at the transcriptional level, we applied actinomycin

Table 2 Genbank identifications, AtPDI features, predicted polypeptide sizes, and predicted presence of signal peptide (s.p.) or other targeting sequences such as chloroplast (chl), mitochondrion (mit), plasma membrane (p.m.) or other

Name AtPDIs	Gene locus accession nos.	Other names	Amino acids	ER ret. signal	Score sig. peptide or other
AtPDI1	At3g54960	AtPDIL1-3	579	KDEL	99% s.p.
	CAB41088				71% chl
AtPDI2	At5g60640	AtPDIL1-4	597	KDEL	100% s.p.
	BAB09837				52% chl
AtPDI3	At1g52260	AtPDIL1-5	537	KDEL	67% s.p.
	AAG51554				
AtPDI4	At3g16110	AtPDIL1-6	534	KDEL	89% s.p.
	BAB02677				40% chl
AtPDI5	At1g21750	AtPDIL1-1	501	KDEL	100% s.p.
	AAD41430				
AtPDI6	At1g77510	AtPDIL1-2	508	KDEL	100% s.p.
	AC010704				
AtPDI7	At4g27080	AtPDIL5-4	480	No	0% s.p.
	T06038				73% other
AtPDI8	At1g35620	AtPDIL5-2	440	KKED	100% s.p.
	AAK62431				88% p.m.
AtPDI9	At2g32920	AtPDIL2-3	440	KDEL	99% s.p.
	AAB91984				
AtPDI10	At1g04980	AtPDIL2-2	443	KDDL	100% s.p.
	AAF40463				61% chl
AtPDI11	At2g47470	AtPDIL2-1	361	No	100% s.p.
	AY091388				
AtPDI12	At3g20560	AtPDIL5-3	483	No	2% s.p.
	NM112948.2				70% other
					53% mit

The numbering of AtPDIs is based on the study of Meiri et al. (2002)

D to block gene transcription prior to inducing ER stress with Tm. The RT-PCR results revealed that, in the presence of transcription inhibitor, the induction of *BiP2*, *AtbZIP60, AtPDI1, AtPDI5, AtPDI6*, *AtPDI9*, *AtPDI10*, and *AtPDI11* genes was significantly (>70%), but not completely, impaired. The effect was most notable at 2 h of Tm treatment, while the induction of some *PDIs* recovered partially at 5 h (Fig. 4). This experiment indicates that the increase in *AtPDI* mRNA levels in plants exposed to the chemical inducers of ER stress occurred in part at the transcription level.

The expression of *AtPDI* genes in *AtIRE1-2* mutants under ER stress

To determine whether the expression of the *AtPDI* genes is controlled by putative ER stress signal transduction pathway components, AtIRE1-1 and AtIRE1-2, we endeavored to identify their homozygous T-DNA insertion mutants. For the *AtIRE1-1* gene, however, only heterozygous mutant plants were recovered after repeated selfing of heterozygous parents and followed by molecular diagnoses of the *ire1-1* mutant (SALK_018150) (supplemental Fig. 2). We used RT-PCR to compare the mRNA levels of the *AtIRE1-1* gene in the *ire1-1* heterozygote relative to wild-type plants (Supplemental Fig. 2d). No difference in *AtIRE1-1* transcript levels was observed.

In contrast to *AtIRE1-1*, we identified three independent homozygous T-DNA insertion lines of the *AtIRE1-2* gene. They were *ire1-2-1* (SALK_010332), *ire1-2-2* (SALK_018112), and *ire1-2-3* (SALK_002316). Sequence analysis of PCR products revealed T-DNAs were inserted in different locations within the *AtIRE1-2* gene (Fig. 5a). The results of RT-PCR using *AtIRE1-2* specific primers confirmed that both *ire1-2-2* and *ire1-2-3* are null mutants (Fig. 5b). Although *ire1-2-1* has transcripts, the levels are much lower level than in wild type (Fig. 5b). To determine the impact of the *AtIRE1-2* mutant on the expression of

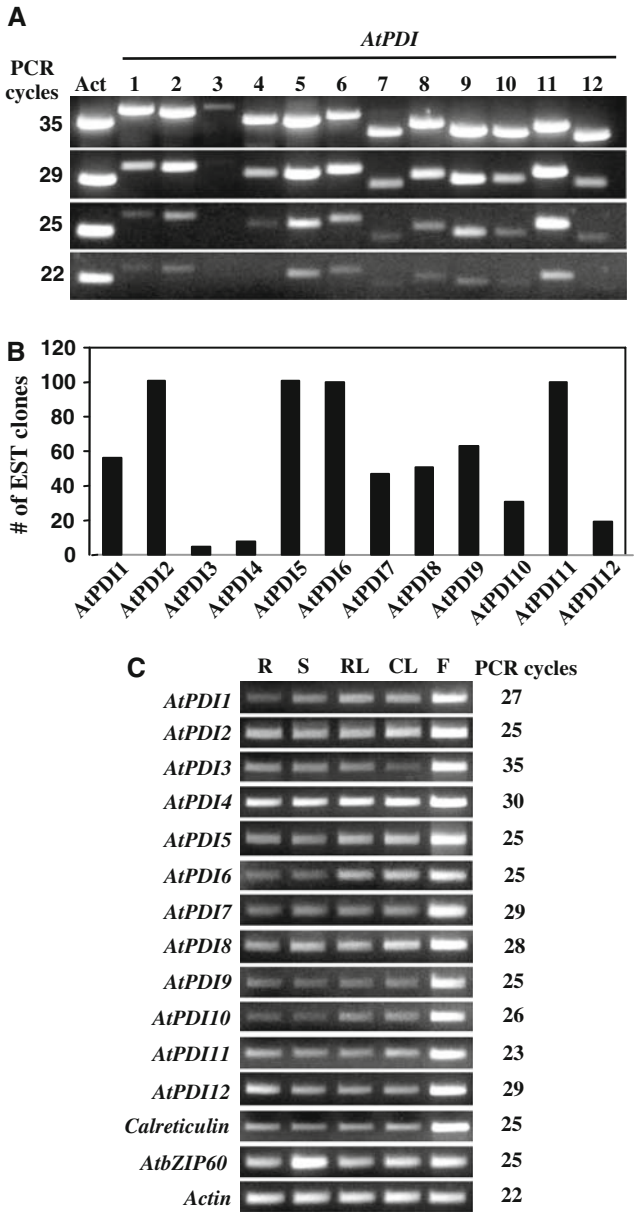

Fig. 2 The relative abundance of *AtPDI* transcripts within seedlings and among tissues. **a** The relative abundance of *AtPDI* transcripts (1–12) and for actin (Act) in 1-week-old liquid-cultured plantlets was determined by RT-PCR analysis for the indicated number of cycles. **b** the number of EST clones of each PDI gene in NCBI EST database is shown. **c** Levels of *AtPDI* mRNAs in different *Arabidopsis* tissues of 2 month-old plants determined by RT-PCR analysis. *R* root, *S* stem, *RL* rosette leaf, *CL* cauline leaf, *F* flower. Actin was used as internal control and the transcript levels for the ER stress genes, encoding calreticulin and AtbZIP60, were also analyzed

AtPDI genes in plants exposed to ER stress, wild type and the three homozygous mutants were treated with Tm (Fig. 5c). Tm treatment induced the expression of six *AtPDI* genes and the two positive controls, *BiP2* and *AtbZIP60*, to comparable levels in the mutant and wild type plants. Therefore, the disruption of *AtIRE1-2* gene did not interfere with Tm-induced *AtPDI* gene expression.

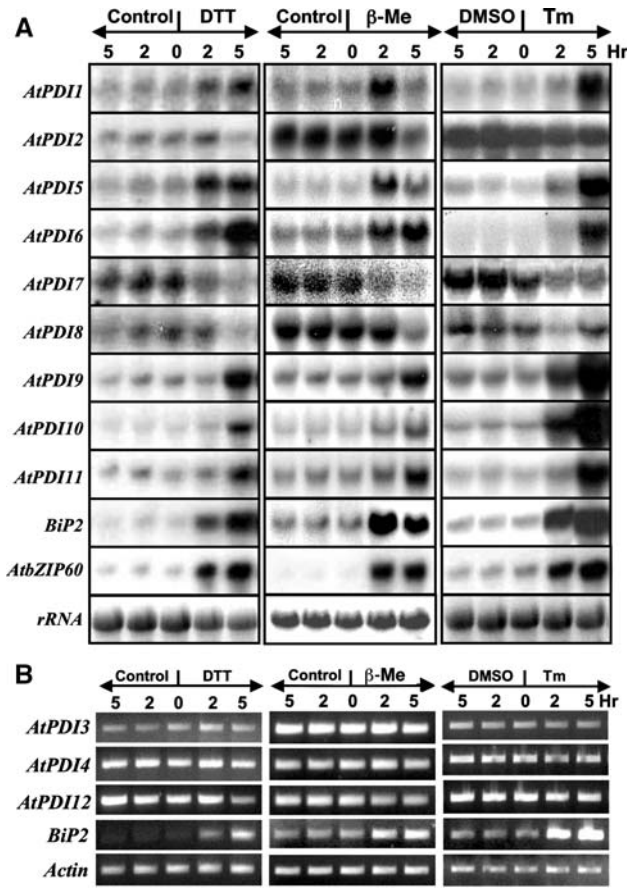

Fig. 3 Levels of the *AtPDI* mRNA in response to treatment with ER-stress inducing chemicals, DTT, β-Me, and Tm, as determined by **a**, RNA gel-blot analysis or **b**, RT-PCR. The liquid-cultured plants were treated with the three chemicals for 0, 2 and 5 h. Analysis of transcripts for the BiP2 and AtbZIP60 genes were used as positive controls for the induction of UPR in response to ER stress. The analysis of rRNA was used as a loading control

The *Atbzip60* mutant affects *AtPDI* gene expression in response to ER stress

AtbZIP60 is reported to be a transcription factor involved in ER stress signaling in plants (Iwata and Koizumi 2005). To test whether AtbZIP60 influences *AtPDI* gene expression in response to ER stress, we identified a homozygous T-DNA insertion in the *AtbZIP60* gene (Fig. 6a). To verify that *AtbZIP60* gene expression was abolished in the *Atbzip60* mutant, RT-PCR analyses were performed on total RNA from mutant and wild type plants using two sets of gene-specific primers (Fig. 6b). No *AtbZIP60* mRNA was detected in the mutant when the forward primer, LP1 (upstream to the T-DNA insertion site), and reverse primer, RP, were used. However, a very faint band was obtained if the primer, RP, and the primer, LP2 (downstream to the T-DNA inserted site), were used (Fig. 6b). This indicated that a truncated *AtbZIP60* mRNA was present in the mutant, although the mechanism of transcription is unknown.

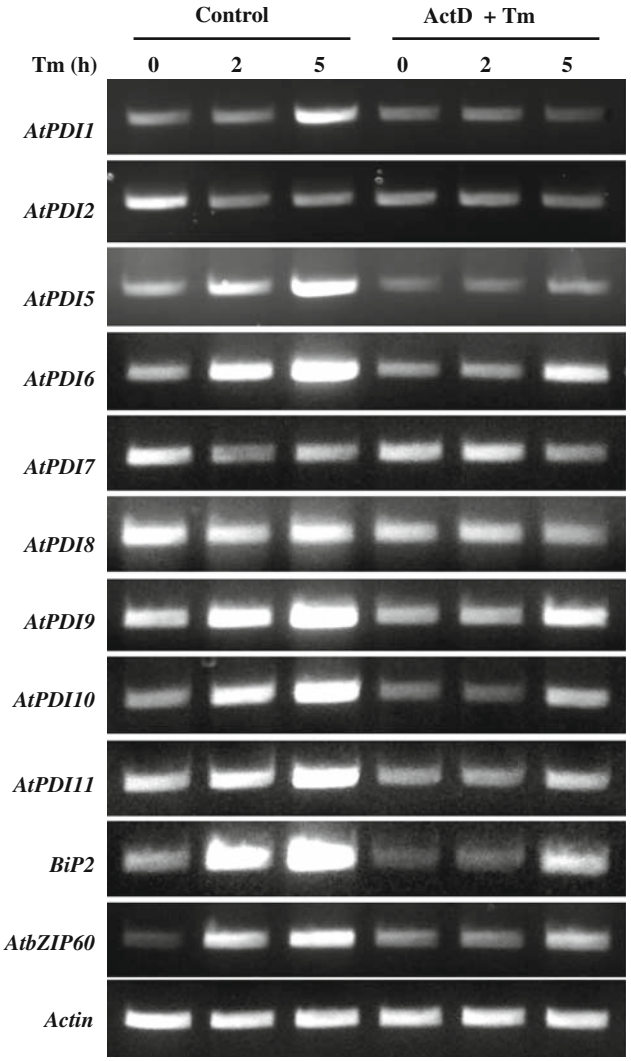

Fig. 4 Determination of the effects of the transcription inhibitor, actinomycin D, on the ability of Tm to induce AtPDI gene expression. RT-PCR analysis was conducted to measure the mRNA levels for key representative *AtPDI* genes, and the control genes, *BiP2*, *AtbZIP60* and actin, in plants exposed to Tm to induce ER stress, after being pre-treated with actinomycin D (ActD)

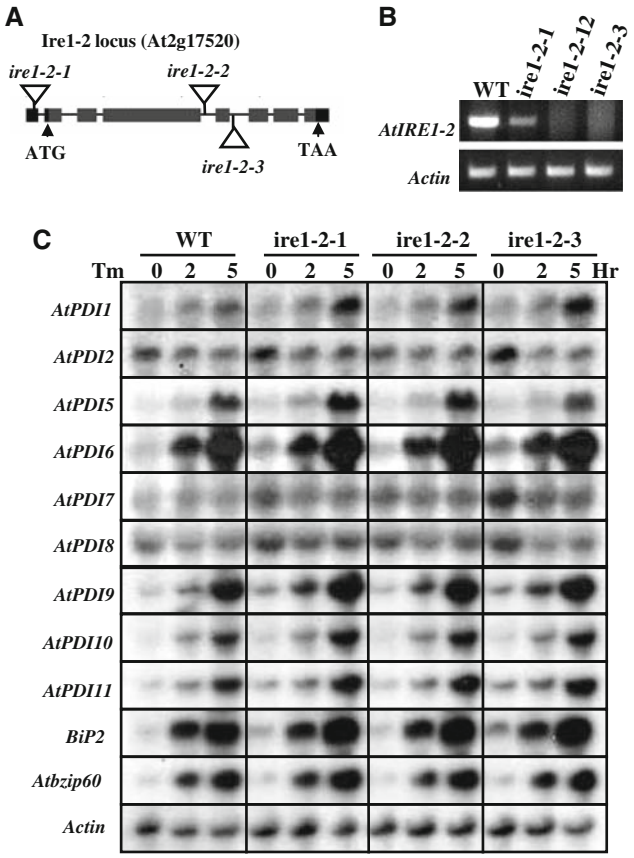

Fig. 5 The effect of disruption of the *AtIRE1-2* gene on the expression of *AtPDI* genes in plants exposed to Tm treatment. **a** the schematic representation of *AtIRE1-2* gene structure and T-DNA insertion sites of three homozygous mutant lines (*ire1-1, ire1-2,* and *ire1-3*). Exons are represented by *grey boxes*, while the UTRs are indicated by *darker boxes*. Introns are represented by *lines*. The positions of the translational start (ATG) and stop (TAA) codons are indicated. In *ire1-2-1*, the T-DNA is inserted at 148 bp upstream from the translation start codon, in the 5′UTR. *Ire1-2-2* has the T-DNA within the fourth intron, at +2018 bp (**a** in ATG is +1). The T-DNA in *ire1-2-3* is inserted at +2329 bp, in the fifth intron (Panel A). **b** the detection of *AtIRE1-2* mRNA in three homozygous mutant lines and wild type by RT-PCR. The actin gene was used as an internal control. **c** RNA gel-blot analysis of *AtPDI* gene expression in the three *AtIRE1-2* mutant lines and wild-type plants exposed to Tm treatment for 0, 2 and 5 h. Transcripts for the BiP2 and AtbZIP60 genes were analyzed as indicators of ER stress

Nevertheless, the experiment confirmed that *AtbZIP60* expression was severely impaired in the mutant (Fig. 6b).

Wild-type and the *Atbzip60* mutant were treated with Tm or β-Me, then the expression of key *AtPDI* genes was analyzed via RNA-gel blot hybridization (Fig. 6c). The expression of the *AtPDI6*, *AtPDI9*, *AtPDI10*, and *AtPDI11* genes decreased in the *Atbzip60* mutant relative to wild type under ER stress treatments, especially at 5 h Tm and β-Me treatments. Thus, the data of 5hr treatment was quantitated and represented as the ratio of RNA levels from mutant compared to wild type plants (Fig. 6d). In particular, the *AtPDI9* mRNA levels in the mutant were 56% after the 5 h Tm treatment and ~34% after the β-Me treatment relative to wild type mRNA levels (Fig. 6c, d). However, Tm and β-Me has moderately opposite effects on *AtPDI1* and *BiP2* expression differed in the *Atbzip60* mutant relative to wild type (Fig. 6c, d). Also, there was a ~20% increase in *AtPDI2* and *AtPDI8* expression in the mutant relative to wild type.

Discussion

Variation in the primary structure and organ expression of the PDI family

The *Arabidopsis* PDI gene family has been proposed to have a varied number of members (Meiri et al. 2002; Lemaire and Miginiac-Maslow 2004; Buchanan and Balmer

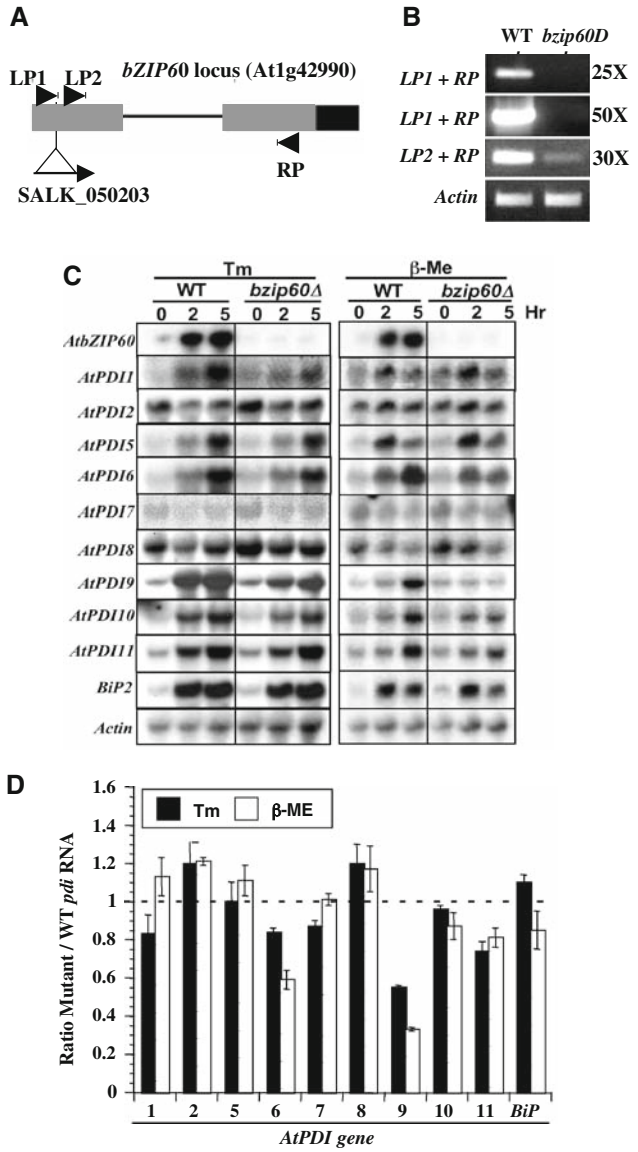

Fig. 6 Analysis of key representative *AtPDI* genes, and the control genes, *BiP2*, *AtbZIP60* and actin, in the homozygous *bzip60* SALK insertion mutant (*bzip60Δ*) and wild-type plants under ER stress. **a** The schematic representation of *AtbZIP60* gene (At1g42990) and SALK T-DNA insertion site. Exons are in *grey rectangles* and intron is a *black line*. A *black rectangle* indicates the 3′ untranslated region. The positions of three *AtbZIP60* gene primers LP1, LP2, and RP are also indicted. LP1 is upstream to the T-DNA insertion site, whereas, LP2 is downstream to the T-DNA insertion site. B, RT-PCR analysis to detect *AtbZIP60* mRNA in the *bzip60Δ* insertion mutant and wild-type plants using different combinations of primers and for 25 and 50 PCR cycles. The actin gene was used as an internal control. **c** RNA gel-blot analysis of *AtPDI* gene expression in the *Atbzip60* mutant and wild-type plants exposed to Tm or *β*-Me treatment for 0, 2 and 5 h. BiP2 gene was used as an indicator of ER stress. **d** Quantitation of the RNA gel-blot analyses of representative *AtPDI* genes and the *BiP2* gene in the *bzip60Δ* mutant relative to wild-type plants exposed to 5 h Tm or *β*-Me treatments. The ratio of RNA levels in the mutant divided by the wild-type were graphed. The actin gene was used as an internal control and set as a value of one (*dashed line*). Values below the *dashed line* indicate a decrease in mRNA levels in the mutant. Values are the means ± standard errors of two independent experiments done in duplicate

2005). Recently, a comprehensive genome-wide search and phylogenetic analysis of proteins that contain thioredoxin domains (thioredoxin superfamily), generated a clade of 22 *Arabidopsis* PDI-like proteins (Houston et al. 2005). However, two of them are quiescin-sulfhydryl oxidases and seven are adenosine 5′-phosphosulfate reductase-like proteins. Here we used a different approach, emphasizing the classical human and yeast PDI domain structure, *a–b–b′–a′* (Wilkinson and Gilbert 2004), to identify 12 *PDI* genes in *Arabidopsis*, and gave weight to amino acid sequence identity beyond the thioredoxin domain, with allowances for minor divergence (Fig. 1; Table 2, Supplementary Fig. 1).

New putative functional domains were identified in the analysis. The12 PDIs differed in polypeptide length, presence of signal peptide and ER retention signal, and the number and positions of TRX and TMD domains. However, key pairs of AtPDIs were very conserved at the amino-acid level. They were AtPDI1 and AtPDI2, AtPDI3 and AtPDI4, PDI5 and PDI6, AtPDI7 and AtPDI12, and AtPDI9 and AtPDI10 (Fig. 1). PDIs are believed to have evolved from an ancestral thioredoxin enzyme (Buchanan 1991; McArthur et al. 2001), and the diverse domain structures generated through duplications and deletions (Kanai et al. 1998; Meiri et al. 2002). Individual PDIs in other eukaryotes vary in their target substrates (McArthur et al. 2001; Wilkinson and Gilbert 2004). The multiplicity of conserved PDIs in *Arabidopsis,* and the structural differences in divergent members, suggest that they could have both specialized and overlapping functions to facilitate adaptation to new biochemical needs or environments during evolution. Alternatively, PDI redundancy provides for genetic compensation, ensuring that mutation or loss of one gene will not have a deleterious impact on the plant. Finally, the overall high level of all *AtPDI* mRNAs in flowers suggests that they play important roles in reproductive development.

ER stress and the UPR regulate the transcription of a sub-group of *AtPDI* genes

We identified six genes (*AtPDI1*, *AtPDI5*, *AtPDI6*, *AtPDI9*, *AtPDI10* and *AtPDI11*) that were up-regulated by UPR in response to well-documented chemical inducers of UPR: DTT, Tm, and *β*-Me (Martinez and Chrispeels 2003; Iwata and Koizumi 2005; Kamauchi et al. 2005). The *BiP2* gene and, in some experiments, the calreticulin gene, served as internal positive controls to confirm that the chemicals were indeed inducing UPR. It is likely that these genes are up-regulated to increase the protein folding capacity in the ER. Such general folding catalysts will have a broader range of substrates, as do BiP and the classical PDI enzyme (Haugstetter et al. 2005). A sub-group of *AtPDI* genes (*AtPDI2*, *AtPDI7*, *AtPDI12*, *AtPDI8*) were not induced by UPR.

There is precedence for a PDI containing an ER-retention signal leaving the ER and entering the chloroplast in *Chlamydomonas* (Levitan et al. 2005). The *Arabidopsis* AtPDI1 and AtPDI2 are most closely related to the *Chlamydomonas* sequence (RB60-PDI). Interestingly, despite their strong sequence similarity between AtPDI1 and AtPDI2, they have different responses to UPR. They may have functionally diverged from each other in terms of gene regulation and protein function.

The use of the transcription inhibitor (actinomycin D) and the promoter disruption mutant demonstrated that ER stress activated transcription of the six *PDI* genes. The inhibitory effect of actinomycin D was not 100%, especially in the longer Tm treatment (5 h, Fig. 4). The concentration of Actinomycin D may be insufficient to block transcription completely, or its activity diminishes during treatment. *AtPDI2* and *AtPDI7* were not induced by Tm, but there was slight changes in mRNA levels within experimental variation.

The AtbZIP60 is partially required for the induction of *AtPDI* genes upon ER stress

We sought to determine whether the induction of *AtPDI* gene transcription was mediated by UPR signal pathway components homologous to those in mammals. To test whether disruption of UPR signaling homologs, AtIRE1-1 or AtIRE1-2 (Koizumi et al. 2001), can affect the up-regulation of *AtPDIs*, we endeavored to identify their homozygous mutants. However, no homozygous *AtIRE1-1* gene T-DNA mutants were present in seed from selfed heterozygous parents. *Atire1-1* homozygosity could be lethal, and that *AtIRE1-1* is an essential gene, however, more evidence is needed to investigate the possible lethality during reproductive development. Coincidently, the deletion of *IRE1-α* in the mouse produced embryonic lethality (Urano et al. 2000b). In contrast to *AtIRE1-1*, we were able to obtain *AtIRE1-2* homozygous null mutants. The induction of *AtPDI* and *BiP2* gene expression was not affected by the disruption of the *AtIRE1-2* gene, which is similar to the response of the *BiP* gene in *IRE1-β* knockout mice (Bertolotti et al. 2001; Urano et al. 2000a). Therefore, AtIRE1-2 might not be involved in regulating *AtPDI* gene expression in response to ER stress in plants, or it is possible that other genes or pathways complement the mutation of the *AtIRE1-2*. In contrast, the *Arabidopsis* ATF6-like protein, AtbZIP60 (Lee et al. 2002; Iwata and Koizumi 2005) was involved in the up-regulation of at least four *AtPDI* genes in response to UPR. The expression of these genes was partially decreased in the *Atbzip60* mutant. We hypothesize that the 20% increase in *AtPDI2* and *AtPDI8* gene expression in the mutant indicated that the AtbZIP60 might also control the expression of repressors of gene expression. The partial and contrasting effects suggest that additional complex UPR signaling components complement activation of PDI gene expression in the *Atbzip60* mutant exposed to ER stress. Recently, a novel ATF6-like transcription factor, AtbZIP28 was identified to mediate UPR in *Arabidopsis*. It appears to be more involved in the early phases of Tm-induced ER stress signaling than AtbZIP60 (Liu et al. 2007b).

Overall, we found that the expression of a sub-group of six *AtPDI* genes was increased in response to ER stress, and the up-regulation was at least partially modulated by AtbZIP60. Our work not only paves the way for extended study of the PDI gene family, but also provides insight into UPR in plants and its relationship with UPR signaling in mammals and yeast. Further research is needed to define the factors and their interaction in the UPR signaling pathway regulating the *AtPDI* genes. The promoters for the UPR-up-regulated *AtPDI* genes will serve as useful tools to begin elucidating the transcriptional regulatory network.

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References

Back SH, Schroder M, Lee K, Zhang K, Kaufman RJ (2005) ER stress signaling by regulated splicing: IRE1/HAC1/XBP1. Methods 35:395–416

Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY (2001) Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. Nat Cell Biol 3:24–29

Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. J Mol Biol 340:783–795

Bertolotti A, Zhang Y, Hendershot LM, Harding HP, Ron D (2000) Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. Nat Cell Biol 2:326–332

Bertolotti A, Wang X, Novoa I, Jungreis R, Schlessinger K, Cho JH, West AB, Ron D (2001) Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. J Clin Invest 107:585–593

Buchanan BB (1991) Regulation of CO_2 assimilation in oxygenic photosynthesis: the ferredoxin/thioredoxin system. Arch Biochem Biophys 288:1–9

Buchanan BB, Balmer Y (2005) REDOX REGULATION: a broadening horizon. Annu Rev Plant Biol 56:187–220

Caramelo J, Castro O, Prat-Gay G, Parodi A (2004) The endoplasmic reticulum glucosyltransferase recognizes nearly native glycoprotein folding intermediates. J Biol Chem 279:46280–46285

Chun L, Kawakami A, Christopher DA (2001) Phytochrome A mediates blue light and UV-A-dependent chloroplast gene transcription in green leaves. Plant Physiol 125:1957–1966

Christopher DA, Mullet JE (1994) Separate photosensory pathways co-regulate blue light/ultraviolet-A-activated *psbD-psbC* transcription and light-induced D2 and CP43 degradation in barley (*Hordeum vulgare*) chloroplasts. Plant Physiol 104:1119–1129

Darby NJ, Kemmink J, Creighton TE (1996) Identifying and characterizing a structural domain of protein disulfide isomerase. Biochemistry 35:10517–1052

Dorner AJ, Wasley LC, Raney P, Haugejorden S, Green M, Kaufman RJ (1990) The stress response in Chinese hamster ovary cells.

Regulation of ERp72 and protein disulfide isomerase expression and secretion. J Biol Chem 265:22029–22034
Edman JC, Ellis L, Blacher RW, Roth RA, Rutter WJ (1985) Sequence of protein disulphide isomerase and implications of its relationship to thioredoxin. Nature 317:267–270
Galtier N, Gouy M, Gautier C (1996) SeaView and Phylo_win, two graphic tools for sequence alignment and molecular phylogeny. Comput Appl Biosci 12:543–548
Gething MJ (1999) Role and regulation of the ER chaperone BiP. Semin Cell Dev Biol 10:465–472
Gruber CW, Cemazar M, Heras B, Martin JL, Craik DJ (2006) Protein disulfide isomerase: the structure of oxidative folding. Trends Biochem Sci 31:455–464
Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D (2000) *Perk* is essential for translational regulation and cell survival during the unfolded protein response. Mol Cell 5:897–904
Harding HP, Calfon M, Urano F, Novoa I, Ron D (2002) Transcriptional and translational control in the Mammalian unfolded protein response. Annu Rev Cell Dev Biol 18:575–599
Haugstetter J, Blicher T, Ellgaard L (2005) Identification and characterization of a novel thioredoxin-related transmembrane protein of the endoplasmic reticulum. J Biol Chem 280:8371–8380
Hoffer PH, Christopher DA (1997) Structure and blue-light-responsive transcription of a chloroplast psbD promoter from *Arabidopsis thaliana*. Plant Physiol 115:213–222
Houston NL, Fan C, Xiang JQ, Schulze JM, Jung R, Boston RS (2005) Phylogenetic analyses identify 10 classes of the protein disulfide isomerase family in plants, including single-domain protein disulfide isomerase-related proteins. Plant Physiol 137:762–778
Iwata Y, Koizumi N (2005) An *Arabidopsis* transcription factor, AtbZIP60, regulates the endoplasmic reticulum stress response in a manner unique to plants. Proc Natl Acad Sci USA 102:5280–5285
Jämsä E, Simonen M, Makarow M (1994) Selective retention of secretory proteins in the yeast endoplasmic reticulum by treatment of cells with a reducing agent. Yeast 10:355–370
Kamauchi S, Nakatani H, Nakano C, Urade R (2005) Gene expression in response to endoplasmic reticulum stress in *Arabidopsis thaliana*. FEBS J 272:3461–3476
Kanai S, Toh H, Hayano T, Kikuchi M (1998) Molecular evolution of the domain structures of protein disulfide isomerases. J Mol Evol 47:200–210
Kanemoto S, Kondo S, Ogata M, Murakami T, Urano F, Imaizumi K (2005) XBP1 activates the transcription of its target genes via an ACGT core sequence under ER stress. Biochem Biophys Res Commun 331:1146–1153
Koizumi N, Martinez IM, Kimata Y, Kohno K, Sano H, Chrispeels MJ (2001) Molecular characterization of two *Arabidopsis* Ire1 homologs, endoplasmic reticulum-located transmembrane protein kinases. Plant Physiol 127:949–962
Kostova Z, Wolf DH (2003) For whom the bell tolls: protein quality control of the endoplasmic reticulum and the ubiquitin-proteasome connection. EMBO J 22:2309–2317
Lee AH, Iwakoshi NN, Glimcher LH (2003) XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. Mol Cell Biol 23:7448–7459
Lee K, Tirasophon W, Shen X, Michalak M, Prywes R, Okada T, Yoshida H, Mori K, Kaufman RJ (2002) IRE1-mediated unconventional mRNA splicing and S2P-mediated ATF6 cleavage merge to regulate XBP1 in signaling the unfolded protein response. Genes Dev 16:452–466
Lemaire SD, Miginiac-Maslow M (2004) The thioredoxin superfamily in *Chlamydomonas reinhardtii*. Photosynth Res 82:203–220
Levitan A, Trebitsh T, Kiss V, Pereg Y, Dangoor I, Danon A (2005) Dual targeting of the protein disulfide isomerase RB60 to the chloroplast and the endoplasmic reticulum. Proc Natl Acad Sci USA 102:6225–6230
Liu JX, Srivastava R, Che P, Howell SH (2007a) Salt stress responses in *Arabidopsis* utilize a signal transduction pathway related to endoplasmic reticulum stress signaling. Plant J 51:897–909
Liu JX, Srivastava R, Che P, Howell SH (2007b) An endoplasmic reticulum stress response in *Arabidopsis* is mediated by proteolytic processing and nuclear relocation of a membrane-associated transcription factor, bZIP28. Plant Cell 19:4111–4119
Lucero HA, Kaminer B (1999) The role of calcium on the activity of ER calcistorin/protein-disulfide isomerase and the significance of the C-terminal and its calcium binding. J Biol Chem 274:3243–3251
Martinez IM, Chrispeels MJ (2003) Genomic analysis of the unfolded protein response in *Arabidopsis* shows its connection to important cellular processes. Plant Cell 15:561–576
McArthur AG, Knodler LA, Silberman JD, Davids BJ, Gillin FD, Sogin ML (2001) The evolutionary origins of eukaryotic protein disulfide isomerase domains: new evidence from the Amitochondriate protist *Giardia lamblia*. Mol Biol Evol 18(8):1455–1463
Meiri E, Levitan A, Guo F, Christopher DA, Schaefer D, Zryd JP, Danon A (2002) Characterization of three PDI-like genes in *Physcomitrella patens* and construction of knock-out mutants. Mol Genet Genomics 267:231–240
Mori K, Ogawa N, Kawahara T, Yanagi H, Yura T (2000) mRNA splicing-mediated C-terminal replacement of transcription factor Hac1p is required for efficient activation of the unfolded protein response. Proc Natl Acad Sci USA 97:4660–4665
Noh SJ, Kwon CS, Chung WI (2002) Characterization of two homologs of Ire1p, a kinase/endoribonuclease in yeast, in *Arabidopsis thaliana*. Biochim Biophys Acta 1575:130–134
Noh SJ, Kwon CS, Oh DH, Moon JS, Chung WI (2003) Expression of an evolutionarily distinct novel BiP gene during the unfolded protein response in *Arabidopsis thaliana*. Gene 311:81–91
Noiva R (1999) Protein disulfide isomerase: the multifunctional redox chaperone of the endoplasmic reticulum. Semin Cell Dev Biol 10:481–493
Okushima Y, Koizumi N, Yamaguchi Y, Kimata Y, Kohno K, Sano H (2002) Isolation and characterization of a putative transducer of endoplasmic reticulum stress in *Oryza sativa*. Plant Cell Physiol 43:532–539
Ron D (2002) Translational control in the endoplasmic reticulum stress response. J Clin Invest 110:1383–1388
Saitou N, Nei M (1997) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
Takatsuki A, Arima K, Tamura G (1971) Tunicamycin, a new antibiotic. I. Isolation and characterization of tunicamycin. J Antibiot (Tokyo) 24:215–223
Tasanen K, Parkkonen T, Chow LT, Kivirikko KI, Pihlajaniemi T (1998) Characterization of the human gene for a polypeptide that acts both as the beta subunit of prolyl 4-hydroxylase and as protein disulfide isomerase. J Biol Chem 263:16218–16224
Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P (2000) Functional and genomic analyses reveal essential coordination between the unfolded protein response and ER-associated degradation. Cell 101:249–258
Trombetta ES, Parodi AJ (2003) Quality control and protein folding in the secretory pathway. Annu Rev Cell Dev Biol 19:649–676
Urano F, Bertolotti A, Ron D (2000a) IRE1 and efferent signaling from the endoplasmic reticulum. J Cell Sci 113:3697–3702
Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D (2000b) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287:664–666

Vincent MJ, Martin AS, Compans RW (1998) Function of the KKXX motif in endoplasmic reticulum retrieval of a transmembrane protein depends on the length and structure of the cytoplasmic domain. J Biol Chem 273:950–956

Wadahama H, Kamauchi S, Ishimoto M, Kawada T, Urade R (2007) Protein disulfide isomerase family proteins involved in soybean protein biogenesis. FEBS J 274:687–703

Wilkinson B, Gilbert HF (2004) Protein disulfide isomerase. Biochim Biophys Acta 1699:35–44

Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K (2001) XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 107:881–891