AtUTr1, a UDP-glucose/UDP-galactose Transporter from *Arabidopsis thaliana*, Is Located in the Endoplasmic Reticulum and Up-regulated by the Unfolded Protein Response

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The folding of glycoproteins in the endoplasmic reticulum (ER) depends on a quality control mechanism mediated by the calnexin/calreticulin cycle. During this process, continuous glucose trimming and UDP-glucose-dependent re-glucosylation of unfolded glycoproteins takes place. To ensure proper folding, increases in misfolded proteins lead to up-regulation of the components involved in quality control through a process known as the unfolded protein response (UPR). Reglucosylation is catalyzed by the ER lumenal located enzyme UDP-glucose glycoprotein glucosyltransferase, but as UDP-glucose is synthesized in the cytosol, a UDP-glucose transporter is required in the calnexin/calreticulin cycle. Even though such a transporter has been hypothesized, no protein playing this role in the ER yet has been identified. Here we provide evidence that AtUTr1, a UDP-galactose/glucose transporter from *Arabidopsis thaliana*, responds to stimuli that trigger the UPR increasing its expression around 9-fold. The accumulation of *AtUTr1* transcript is accompanied by an increase in the level of the AtUTr1 protein. Moreover, subcellular localization studies indicate that AtUTr1 is localized in the ER of plant cells. We reasoned that an impairment in *AtUTr1* expression should perturb the calnexin/calreticulin cycle leading to an increase in misfolded protein and triggering the UPR. Toward that end, we analyzed an *AtUTr1* insertional mutant and found an up-regulation of the ER chaperones *BiP* and *calnexin*, suggesting that these plants may be constitutively activating the UPR. Thus, we propose that in *A. thaliana*, AtUTr1 is the UDP-glucose transporter involved in quality control in the ER.

The proper folding and assembly of proteins in the endoplasmic reticulum (ER)[6] is maintained by quality control mechanisms that avoid the release of misfolded proteins by retaining them within the organelle. Most of the proteins synthesized in the ER are glycoproteins that contain the oligosaccharide $Glc_3Man_9GlcNAc_2$. This structure is co-translationally transferred to the protein and immediately trimmed to $GlcMan_9GlcNAc_2$ (1–3). The chaperones calnexin and calreticulin bind this monoglucosylated form retaining the glycoprotein in the ER, facilitating its folding (4). To release the protein from the chaperone, the glucose residue is removed by glucosidase II, and if the protein is correctly folded, it can exit the ER (2, 5). However, if the protein is misfolded, it is recognized by UDP-glucose glycoprotein glucosyltransferase (UGGT) and reglucosylated, thereby allowing its re-association with calnexin and calreticulin (6–9). Therefore, UGGT plays an essential role in quality control by allowing the re-entry of the glycoprotein into the folding cycle.

UGGT is located in the lumen of the ER and utilizes UDP-glucose as substrate. However, UDP-glucose is synthesized in the cytosol and needs to be translocated into the lumen. The protein involved in this process likely belongs to the family of nucleotide sugar transporters (NSTs). Although several NSTs have been described in eukaryotes (10), most of them have been related to protein glycosylation and polysaccharide biosynthesis in the Golgi apparatus and no NST involved in supplying the substrate for UGGT has yet been described.

Under certain circumstances there is an accumulation of unfolded proteins in the ER which triggers a mechanism known as the unfolded protein response (UPR). During this process, the expression of a large set of genes, in particular chaperones involved in quality control, is induced (11–13). Functional genomic analysis of the UPR in *Arabidopsis thaliana* showed an increase in the expression of genes related to protein folding, glycosylation, vesicle trafficking, and protein degradation (14, 15). The biggest group of up-regulated genes is ER chaperones such as *BiP*, protein-disulfide isomerase (PDI), *calnexin*, and *calreticulin*. Interestingly, microarray analysis showed that one of the up-regulated genes corresponded to *AtUTr1*, a nucleotide sugar transporter.

Norambuena *et al.* (16) identified and characterized AtUTr1, a nucleotide sugar transporter from *A. thaliana*. Upon expression in yeast, AtUTr1 was able to transport UDP-galactose and UDP-glucose, with the transport of UDP-glucose being 200 times higher than UDP-galactose. In addition, analysis of the AtUTr1 protein sequence shows a KK*XX* motif in the C terminus, a sequence that has been described as an ER retention signal for membrane proteins (17). Based on these findings we hypothesized that AtUTr1 may be involved in quality control in the ER. To provide evidence supporting this hypothesis, we exposed *Arabidopsis* plants to a treatment that induced the UPR and then performed a quantitative analysis of the *AtUTr1* transcript. The results showed an accumulation of both the *AtUTr1* transcript and protein upon induction of the UPR. To analyze whether the location of this nucleotide sugar transporter was consistent with a role in quality control, its sub-

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[6] The abbreviations used are: ER, endoplasmic reticulum; UGGT, UDP-glucose glycoprotein glucosyltransferase; NST, nucleotide sugar transporter; UPR, unfolded protein response; GFP, green fluorescent protein; YFP, yellow fluorescent protein; DTT, dithiothreitol; RGP, reversibly glycosylated protein.

cellular distribution was assessed using a GFP-tagged protein and immunodetection upon subcellular fractionation. The results of both approaches are consistent with an ER location of AtUTr1. Furthermore, if AtUTr1 plays a role in supplying the substrate to UGGT, a lack of its expression may perturb the calnexin/calreticulin cycle leading to an accumulation of misfolded protein and triggering the UPR. Toward this end we characterized an *Arabidopsis* insertional mutant in the *AtUTr1* gene that shows less incorporation of UDP-glucose into ER vesicles and observed that the expression level of the chaperones *BiP* and *calnexin* are constitutively up-regulated, while the expression of other genes is not. Thus we propose that AtUTr1 is a UDP-glucose transporter that provides the substrate for UGGT, forming part of the quality control mechanism present at the ER.

EXPERIMENTAL PROCEDURES

Plant Growth Condition—Sterile seeds of wild type and *atutr1* mutant (ecotype Landsberg-erecta) were germinated in a 16 h light/8 h dark cycle at 23 °C in Murashige and Skoog medium (MS) containing 1% sucrose (w/v). For the UPR studies, plants were grown as described by Martínez and Chrispeels (14). Seeds were germinated in 13 ml of liquid MS medium and cultured with gentle shaking for 6 days. The seedlings were treated for 5 h with or without 10 mM of dithiothreitol (DTT). For subcellular fractionation and RNA expression analysis, plants were grown in soil. Agrobacterium-mediated infiltration was performed in *Nicotiana tabacum* (Xanthi NN) leaves.

Cloning of AtUTr1-GFP—AtUTr1 cDNA was amplified by PCR from the *A. thaliana* cDNA library pFL61 (American Type Culture Collection) using platinum *Pfx* polymerase (Invitrogen) and primers flanking the coding region, designed from the genomic sequence. The sequence of the forward primer was 5′-TCTAGGATCCTAATGGAGGTCCATGGCTCC-3′ containing a BamHI restriction site (underlined sequence). The reverse primer was 5′-AGGGAAGATCTCTCCACTCTTTTGCTTCTTCTT-3′ containing a BglII restriction site (underlined sequence) deleting the original stop codon. A unique amplification product of the expected size (1 kb) was obtained. The PCR product was sequenced and digested with BamHI and BglII. The DNA fragment was cloned in the plant expression vector pCD3–326 to generate pCD3–326 AtUTr1-GFP. The AtUTr1-GFP fusion was excised using BamHI and SacI and cloned in the binary vector pBEL-103.

Tobacco Leaf Infiltration and Visualization—Tobacco leaves were infiltrated with AtUTr1-GFP, GONST1-YFP (18), HDEL-GFP (19), and soluble GFP, as described by Kato *et al.* (20). Transformed leaves were examined 2 days after infiltration by epifluorescence microscopy (Olympus IX70) and confocal microscopy (Zeiss LSM 510 Meta).

Isolation and Characterization of A. thaliana Membranes—Caulinar leaves obtained from 4-week-old plants were homogenized and subjected to subcellular fractionation as described by Muñoz *et al.* (21). Membrane fractions were taken from the 1.1/0.25 and 1.3/1.1 M sucrose interfaces and used to measure Golgi and ER markers respectively. Reversibly glycosylated protein (RGP) (22) and xyloglucan fucosyltransferase (23) were used as Golgi markers and NADH cytochrome *c* reductase insensitive to antimycin A as ER marker (24). Protein concentration was determined using the BCA method according to the manufacturer's instructions (Pierce). The distribution of RGP and xyloglucan fucosyltransferase was determined by Western blot using 30 μg of protein separated by SDS-PAGE and transfered onto polyvinylidene difluoride membrane. The immunoblot was performed using a polyclonal antisera raised against RGP or xyloglucan fucosyltransferase (kindly donated by K. Dhugga and W. D. Reiter, respectively) following standard procedures (25). The endoplasmic reticulum marker, NADH cytocrome *c* reductase, insensitive to antimicyn A was measured as described by Briskin *et al.* (26).

Immunodetection of AtUTr1—A synthetic peptide (YQIYLKWKKLQRVEKKKQKS) from the C-terminal sequence of AtUTr1 was synthesized by Bio-Synthesis Inc., coupled to Blue Carrier, and used to immunize rabbits. The antiserum obtained was purified using the synthetic peptide coupled to a cyanogen bromide-Sepharose-activated matrix (Sigma), according to the manufacturer's instructions. The affinity-purified antibody was characterized by enzyme-linked immunosorbent assay and was able to detect in the Western blot a single band of the expected size. For Western blot analysis, 30 μg of proteins from subcellular fractions, or from plants treated with DTT, were denatured in Laemmli buffer at 60 °C for 20 min, separated by SDS-PAGE, and transferred onto polyvinylidene difluoride membrane. Actin was used as a control for protein loading in Western blot analysis.

Isolation of RNA and Northern Blot Analysis—Total RNA from plantlets and flowers was extracted using TRIzol (Invitrogen). Total RNA (20 μg) was fractionated by electrophoresis in an agarose gel containing formaldehyde and capillary transferred to nylon membranes (Hybond N+; Amersham Biosciences) using 10× SSC. Hybridization and all the other procedures were done as described by Orellana *et al.* (27). A specific ^{32}P-radiolabeled probe against *AtUTr1* was prepared from the 3′-untranslated region by PCR. An *AtUTr1* EST (APD06f05) kindly donated by E. Asamizu was used as template. The forward primer was 5′-CAAAAGAGCTCAAGTTTTTCCCTC-3′, and the reverse primer was 5′-TTTGGGTACCCCAAAATTCAAATC-3′. The DNA probes for *BiP*, *calnexin*, and *AtUTr2* were generated by random priming of PCR fragments using an oligolabeling kit (Fermentas). The amplicons for *BiP*, *calnexin*, and *AtUTr2* were produced using the following primers: *BiP*, 5′-ATGGCTCGCTCGTTTGGAGC-3′ (forward) and 5′-AAGTTTCCTGTCCTTTTGAA-3-′ (reverse), *calnexin*, 5′-ATGAGACAACGGCAACTATT-3′ (forward) and 5′-TTCCTGAGGACGGAGGTACT-3′ (reverse); *AtUTr2*, 5′-CACATTTATCGGTCAAGTCTCCGTT-3′ (forward) and 5′-TCGCAGGAGGCGATGGTGATAGAGAAGA-3′ (reverse).

Real-time PCR—mRNA content for *AtUTr1*, *calnexin*, *BiP*, and *AtUTr2* was quantified using the LightCycler system (Roche Diagnostic). Real-time PCR reactions were run in 20 μl final volume and contained 50 ng of cDNA, 1× Master CYBR Green mix, 1.5 mM $MgCl_2$, and a 0.5 μM concentration of each primer. The primers used were the same described above. cDNA was obtained by reverse transcription of 1 μg of total RNA isolated from plants treated with or without 10 mM DTT. The absolute standard curves for each gene were generated by diluting the plasmid carrying the gene of interest from 10,000 to 0.01 pg. The threshold cycle number (C_T) from each sample was referred to the absolute standard curve to estimate the content of the corresponding cDNA. The amount for each cDNA was normalized against the amount of the *actin* cDNA estimated on each sample. Experiments were performed in triplicate.

AtUTr1 Mutant Identification and Characterization—A gene trap line containing a transposon insertion (Ds element) in the first exon of the *AtUTr1* gene was identified from the Sundaresan collection (28, 29). The seeds were obtained from the Nottingham Arabidopsis Stock Center. The Ds insertion was confirmed by PCR using primers for the *AtUTr1* gene (5′-ATGCAAGATGCTCGAACC-3′) and the left border of the Ds element (5′-ACGGTCGGGAAACTAGCTCTAC-3′). For Southern blot analysis 4 μg of genomic DNA were digested using EcoRI, BglII, and HindIII. The fragments were fractionated by electrophoresis in a 0.8% agarose gel and then denaturated and neutralized. The hybridization was performed at 65 °C using an *AtUTr1* or a *GUS* probe

(cDNAs) radiolabeled by random priming oligolabeling kit (Fermentas). The genomic DNA was prepared as described by Silva *et al.* (30).

UDP-glucose Uptake Assays in ER-derived Vesicles—ER vesicles were obtained as described above. For the uptake assays, 50 μg of protein corresponding to the ER vesicles from the wild type and *atutr1* plants were incubated with 1 μM UDP-[^{3}H]glucose (0.1 μCi) in a medium containing 0.25 M sucrose, 10 mM Tris-HCl, pH 7.5, and 1 mM $MgCl_2$ (STM buffer) for 3 min. To stop the reaction, the vesicles were diluted in cold STM buffer and filtered through 0.7-μm glass fiber filters. Subsequently the filters were washed with 10 volumes of cold STM buffer and dried, and the radioactivity was determinated by liquid scintillation counting.

Co-expression Analysis of AtUTr1, BiP, and Calnexin—The accession number for each gene (At2g02810, At5g42020, and At5g61790, respectively) was used to perform a co-expression analysis using the *A. thaliana* co-response data base module of CSB.DB. The matrix utilized contains all the genes analyzed for a set of abiotic stress experiments. The results are grouped automatically by function using the MAPMAN program.

RESULTS

AtUTr1 Responds to the UPR—We analyzed the expression of the *AtUTr1* gene in 6-day-old plants treated with DTT, a treatment that is known to trigger the UPR given its interference in the formation of disulfide bonds (31). Northern blot analysis showed that plants treated with DTT increased the expression of *AtUTr1* as well as the expression of the chaperones *BiP* and *calnexin*, two genes up-regulated under this treatment (32, 33) (Fig. 1*A*). The expression of *actin* showed no change, confirming that the treatment was specific. To obtain more accurate and quantitative information of the changes in *AtUTr1* transcript levels caused by DTT, we performed real-time PCR analysis. The results of this experiment showed a 9-fold increase in the *AtUTr1* transcript amount in plants exposed to DTT (Fig. 1*B*), confirming the result obtained by Northern analysis.

To study whether the changes in transcript levels correlated with an increase in the accumulation of the protein, we performed Western blot analysis using an AtUTr1 peptide affinity-purified antibody. The results showed that seedlings treated with DTT had an increase in the amount of the AtUTr1 protein whereas actin levels did not change under these conditions (Fig. 1*C*). All these results strongly suggest that AtUTr1 is an UPR up-regulated gene.

Expression of AtUTr1 Is Related to Genes Involved in Protein Folding—In plants grown under different conditions, the analysis of genes that show the same profile of expression as the *AtUTr1* gene may provide information regarding the cellular processes in which they are involved. Using the data base module of CSB.DB (34) and genome chip expression profiles obtained from different plant growth condition (35), a co-expression analysis was carried out. The results indicate that expression of *AtUTr1* strongly correlates with the expression of *BiP*, *calnexin*, and other chaperones or proteins related to stress (data not shown). In addition, the largest set of genes co-expressing with *AtUTr1*, *BiP*, and *calnexin* at the same time correspond to proteins related to stress and redox regulation (Fig. 2). As a control, the expression of *AtUTr2*, a Golgi-localized UDP-galactose transporter (36), was analyzed. However, its expression pattern did not show any correlation with the expression of genes encoding for proteins involved in protein folding (data not shown).

AtUTr1 Is Localized at the Endoplasmic Reticulum—Since AtUTr1 transports UDP-glucose (16) and responds to the UPR, we hypothesized that this protein could be in the ER. To investigate its subcellular location, we constructed a chimera by fusing the green fluorescent protein

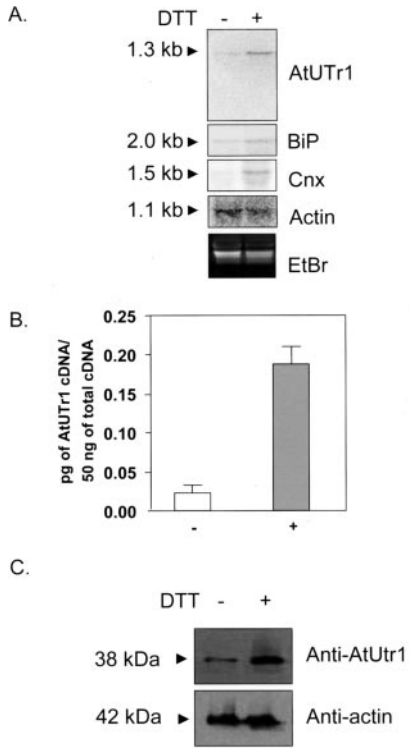

FIGURE 1. ***AtUTr1* mRNA expression and protein accumulation in DTT treated seedlings.** Seedlings were grown in liquid culture for 6 days and then treated for 5 h with 10 mM DTT. *A*, 20 μg of total RNA obtained from control (−) or treated plants (+) was fractionated, transferred to a nylon membrane, and hybridized with a ^{32}P-labeled specific probe against *AtUTr1*, *calnexin* (*Cnx*), *BiP*, or *actin*. *B*, the amount of *AtUTr1* transcript (mean ± S.D.) was determined by real-time PCR in control plants and plants treated with 10 mM DTT as described above. The determinations were performed as described under "Experimental Procedures." *C*, 30 μg of total protein extracted from control (−) or treated (+) plants were subjected to Western blot analysis. AtUTr1 was detected using an affinity-purified peptide antibody. Actin was used as a loading control.

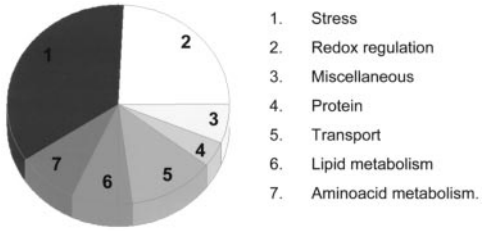

FIGURE 2. ***AtUTr1* co-expression analysis.** The expression of *AtUTr1* was analyzed using the *A. thaliana* co-response data base. An intersection gene query for *AtUTr1*, *BiP*, and *calnexin* was used. The *pie chart* illustrates the categories in which the genes were grouped, corresponding to a common co-response with all three genes.

to the C terminus of AtUTr1. This chimeric protein was capable of incorporating UDP-galactose into microsomes from tobacco leaves infiltrated with AtUTr1-GFP, indicating that the fusion protein was functional. Since most of the nucleotide sugar transporters identified to date are localized in the Golgi apparatus, the distribution of AtUTr1-GFP expressed in epidermal cells of tobacco leaves was compared with the pattern of both ER and Golgi markers. The distribution of soluble GFP was also analyzed (Fig. 3*A*). The results showed that AtUTr1-GFP has a reticulated distribution (Fig. 3*D*), resembling that obtained for GFP-HDEL, an ER located protein (Fig. 3*B*). In contrast, GONST1-YFP, a Golgi-localized GDP-mannose transporter (18), showed a punctate pattern (Fig. 3*C*). The fluorescent dots were motile and sensitive to brefeldin A (data not shown). These results suggest that AtUTr1 is located at the ER and not at the Golgi apparatus.

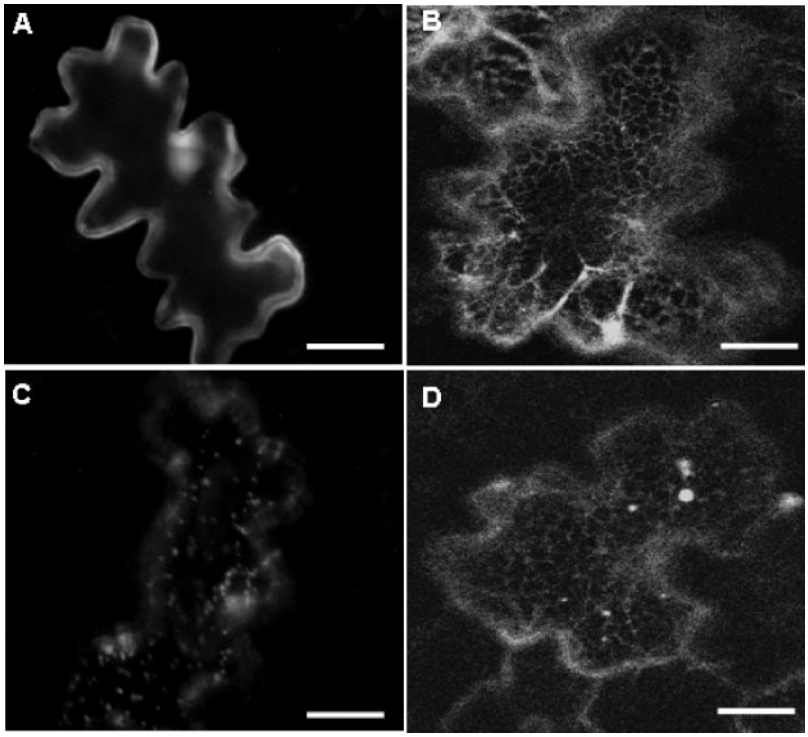

FIGURE 3. **Distribution pattern of AtUTr1-GFP in tobacco leaves.** Epidermal cells from tobacco leaves were infiltrated with *A. tumefaciens* cultures transformed with different constructs. *A*, soluble GFP; *B*, HDEL-GFP (ER marker); *C*, GONST1-YFP (Golgi marker); *D*, AtUTr1-GFP. After 2 days they were analyzed by epifluorescence microscopy. The *bar* corresponds to 20 μm.

To confirm the localization of AtUTr1, we performed subcellular fractionation of *Arabidopsis* plants and analyzed the location of AtUTr1 using an affinity-purified peptide antibody raised against the C terminus of AtUTr1. Upon homogenization of the tissue, the organelles were separated on a discontinuous sucrose gradient, and fractions enriched both in ER and Golgi membranes were obtained. The signal detected by the antibody against AtUTr1 (Fig. 4*A*) showed the same distribution as the ER marker (Fig. 4*C*), distinct from the distribution of the two Golgi markers (Fig. 4*B*). These results, along with those obtained using the AtUTr1-GFP fusion protein, strongly suggest that AtUTr1 is indeed located at the ER.

An AtUTr1 Insertional Mutant (atutr1) Shows Up-regulation of UPR-related Genes—If AtUTr1 provides the UDP-glucose required for the re-glucosylation of misfolded glycoproteins, the absence of AtUTr1 should produce an alteration in the normal folding process in the ER. Thus, an increase in misfolded proteins may occur, triggering the UPR. To investigate this hypothesis we searched for mutants in *AtUTr1*. A gene trap line containing an insertion of the Ds element in the *AtUTr1* gene was characterized (Fig. 5*A*) (28, 29). The analysis of this line using PCR indicated that the Ds insertion was in the first exon of the *AtUTr1* gene. Southern analysis showed changes in the pattern of *atutr1* genomic DNA digested with EcoRI, consistent with the insertion of the Ds element in this region (data shown as supplemental Fig. 1). In addition, PCR analysis and the observation that 100% of the plants obtained upon self-pollination were resistant to kanamycin, the selection marker, indicated that the *AtUTr1* insertion line was homozygous (data not shown). The analysis of the number of insertions by Southern blot analysis, detecting the *GUS* gene present in the Ds element, revealed a single band indicating that a single insertion was present in *atutr1* (data shown as supplemental Fig. 1). Furthermore, the F2 population obtained from the backcross showed that 72% of the plants were able to grow in kanamycin, whereas 28% of the plants were sensitive to the antibiotic. This segregation ratio, together with the molecular analysis indicated the presence of a single Ds insertion in the genome of *atutr1*. Northern blot analysis showed that *AtUTr1* was not expressed in the *atutr1* mutant

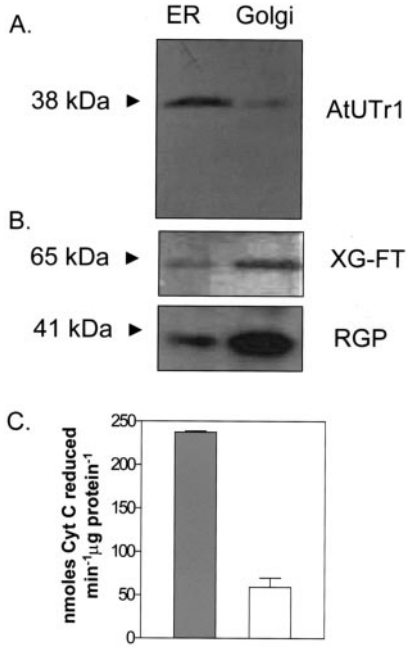

FIGURE 4. **Distribution of AtUTr1 in subcellular membrane fractions from *Arabidopsis* plants.** *Arabidopsis* plants were homogenized and the organelles separated by a discontinuous sucrose gradient. Fractions containing membranes enriched in ER and Golgi were subjected to Western blot analysis using AtUTr1 affinity-purified peptide antibodies (*A*), xyloglucan fucosyltransferase (*XG-FT*), and RGP (Golgi markers) (*B*). As an ER marker the activity of NADH cytochrome *c* reductase insensitive to antimycin A was measured (*C*).

(Fig. 5*B*). Finally, UDP-glucose uptake assays were performed in ER-derived vesicles obtained from wild type and *atutr1* plants (Fig. 5*C*). The result showed that ER-derived vesicles obtained from *atutr1* plants had a decreased uptake of UDP-glucose compared with the wild type.

To test the hypothesis that the UPR could be activated in the *atutr1* mutant, we analyzed the expression of *BiP*. Northern analysis showed that the *BiP* transcript was much more abundant in the *atutr1* mutant

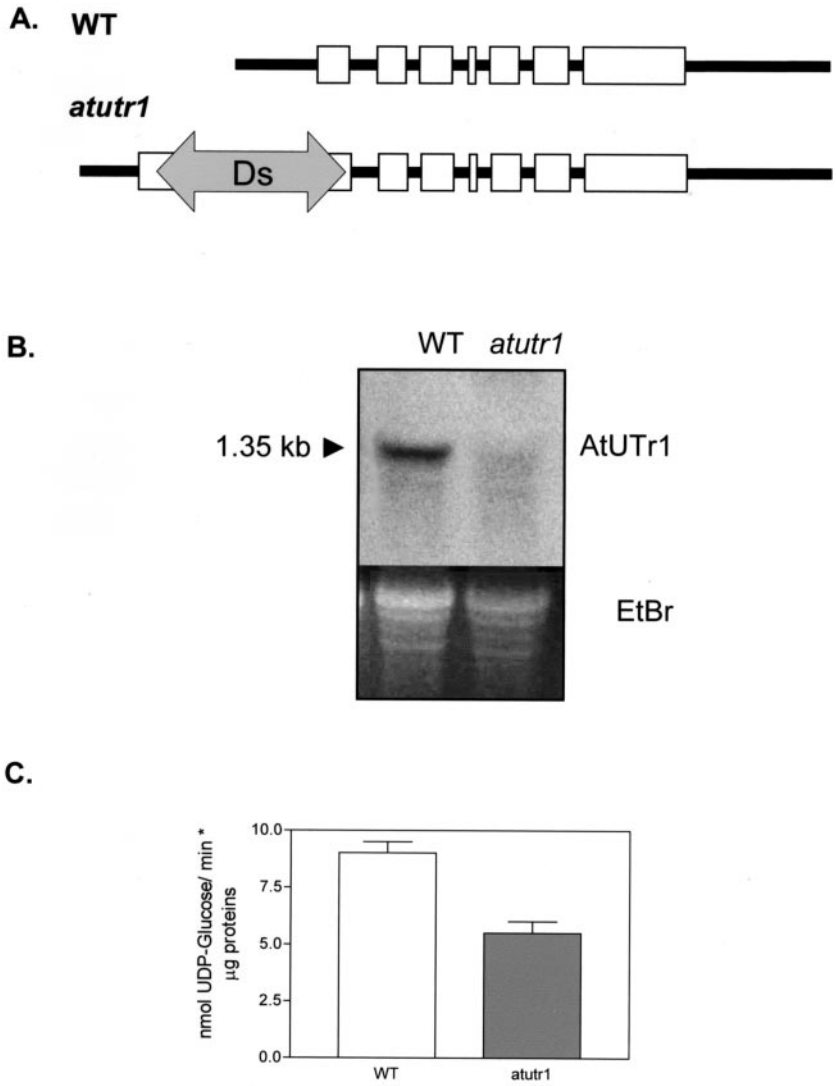

FIGURE 5. **Arabidopsis AtUTr1 mutant plant.** *A*, schematic representation of the *AtUTr1* gene structure. The figure shows the insertion site of the Ds element in the first exon of the gene. *B*, 40 μg of total RNA of wild type (*WT*) and *atutr1* flowers were fractionated on a formaldehyde-agarose gel, transferred to a nylon membrane, and hybridized with a specific ^{32}P-labeled *AtUTr1* probe as described under "Experimental Procedures." Ethidium bromide staining was used to estimate the amount of RNA on each lane (*lower panel*). *C*, uptake of UDP-[^{3}H]glucose into ER vesicles from *atutr1* and wild type (*WT*) plants. ER vesicles were incubated with 1 μM UDP-[^{3}H]glucose for 3 min. The reaction was stopped by a 10-fold dilution with STM buffer and filtering immediately. Filters were dried, and the radioactivity was counted using liquid scintillation. Results are presented as mean $\pm$ S.D.

whereas the level of the *actin* transcript or the UDP-galactose transporter *AtUTr2* transcript was similar in wild-type and mutant plants (Fig. 6*A*). To quantify the magnitude of the changes in gene expression of chaperones involved in quality control, we carried out real-time PCR experiments. The results showed that *BiP* was 2.8-fold and *calnexin* 1.9-fold more abundant in the *atutr1* mutant compared with the wild type plants (Fig. 6*B*). In contrast, the level of the *AtUTr2* transcript was the same in both the mutant and the wild type. Thus, *BiP* and *calnexin* are overexpressed in the mutant, suggesting that *atutr1* plants have the UPR constitutively activated.

DISCUSSION

The results presented in this paper show that AtUTr1 is located in the endoplasmic reticulum and is up-regulated under conditions that induce the UPR. In addition, an *Arabidopsis* mutant in the AtUTr1 gene exhibits a decreased incorporation of UDP-glucose into the ER and up-regulation of chaperones involved in quality control. This is the first report of a nucleotide sugar transporter located at the ER and linked to the UPR. Hence, we propose that AtUTr1 is a UDP-glucose transporter involved in quality control at the ER.

Quality control of glycoproteins in the ER depends on their retention within the organelle by calnexin and calreticulin (recently revised (see Ref. 46)). During this process glycoproteins undergo a continuous glucosylation/deglucosylation of the $Man_9GlcNAc_2$ oligosaccharide linked to asparragine. A key player in this process is UGGT, the enzyme that recognizes unfolded glycoproteins and transfers glucose from UDP-glucose to the $Man_9GlcNAc_2$ oligosaccharide. The glucosylated glycoprotein is then retained by calnexin and calreticulin until glucosidase II cleaves off the glucose, releasing it from these lectins. If the released glycoprotein is not completely folded, UGGT adds glucose again to the oligosaccharide, maintaining this cycle until the glycoprotein is properly folded (37). Most of the members of the calnexin/calreticulin pathway have been characterized in *Arabidopsis* (38–41) suggesting that the ER quality control process for glycoproteins may occur as in other eukaryotes. To date, no UGGT activity has been reported in plants. However, a gene showing high similarity to UGGT genes from other species exists in *Arabidopsis* suggesting that a functional ortholog may be present in this plant.

UGGT is located in the lumen of the ER and uses UDP-glucose as substrate (7). However, this nucleotide sugar is synthesized in the cytosol, so it has to be transferred into the ER lumen. Until now, no evidence has been provided for transport of UDP-glucose into the ER in plants. However, in *Saccharomyces cerevisiae* transport of UDP-glucose has been measured (42). In addition, *ScHut1*, a gene encoding for a UDP-galactose transporter located in the ER, has been identified in *S. cerevisiae*. Interestingly, *AtUTr1* and *ScHut1* are orthologues. However,

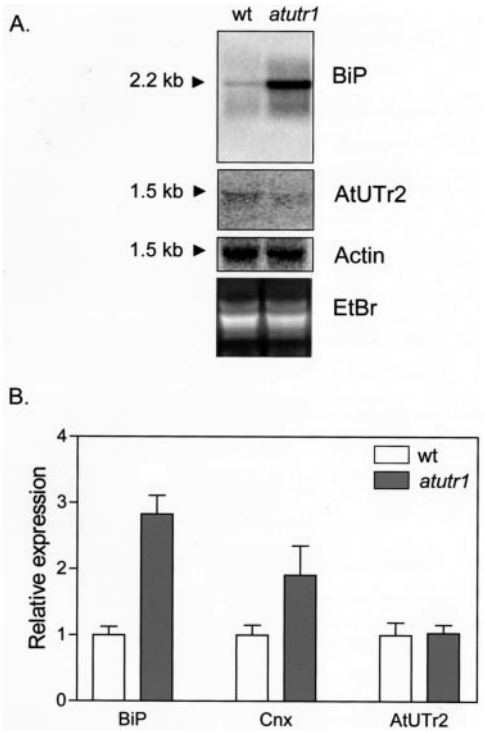

FIGURE 6. **Expression of the ER chaperones in *atutr1* plants.** *A*, 20 μg of total RNA from *atutr1* and wild type flowers were fractionated on a formaldehyde-agarose gel, transferred to a nylon membrane, and hybridized with ^{32}P-labeled probes against *BiP*, *AtUTr2*, and *actin*. *B*, quantification of the amount of transcripts for *BiP*, *calnexin*, and *AtUTr2* in wild type (*wt*) and *atutr1* plants using real-time PCR. The results (mean ± S.D.) are expressed as a ratio between the amount of BiP, calnexin, and AtUTr2 *versus* the amount of the actin transcript, which was the same both in the mutant and wild type.

there is currently a lack of evidence for the presence of UGGT in *S. cerevisiae*, so it is likely that glycoproteins do not go through the calnexin/calreticulin cycle in this organism. Thus, *ScHut1* may not play a role in quality control in this organism. In contrast, the calnexin/calreticulin cycle is active in *Schizosaccharomyces pombe* and a gene with 30–35% identity to *ScHut1* is present in its genome. Curiously, a mutant in this gene (SpHut1) is unable to survive under stress conditions such as in a reducing environment. However, the precise function and subcellular localization of SpHut1 are unknown (43).

The fact that AtUTr1 transports UDP-glucose and is located at the ER makes it a good candidate for being involved in the deliver of UDP-glucose during quality control of glycoproteins in the ER. In addition, the up-regulation of AtUTr1 in conditions that trigger the accumulation of misfolded proteins suggests that the influx of UDP-glucose into the ER is an important step in quality control. Moreover, it also suggests that the incorporation of this nucleotide sugar into the ER is limited by the transporter rather than the availability of substrate in the cytosol.

Recent functional genomic studies using microarrays have indicated that *AtUTr1* is up-regulated by the UPR along with several other genes (14, 15). Thus, our quantitative analysis is in agreement with the microarray data. When we looked for regulatory motifs, we found that the *AtUTr1* promotor region contains the cis element ERSE (endoplasmic reticulum stress element) CC-N$_{12}$-CCACG, which is recognized by AtbZIP60, a transcription factor described as part of the transduction pathway involved in the ER stress response (44). The functional genomic data available also helped us to provide additional evidence for the role of AtUTr1 in quality control. Thus, an analysis of gene co-response data, including the results of a number of microarray experiments aimed at pinpointing changes in gene expression upon abiotic stress, indicated that *AtUTr1* co-expressed mainly with ER chaperones and genes related to stress. These results provide further support for AtUTr1 playing a role in the protein folding process in the ER.

Additional evidence for the role of AtUTr1 in quality control came from our studies in the *Arabidopsis* insertional knock-out mutant. Since the results showed that *atutr1* mutant plants incorporate less UDP-glucose into ER vesicles, we hypothesized that a decrease in the delivery of UDP-glucose into the ER lumen may alter the UGGT/calnexin-calreticulin cycle, leading to an accumulation of unfolded glycoproteins, resulting in activation of the UPR. The expression of both *BiP* and *calnexin* was up-regulated in the mutant, whereas the expression of *AtUTr2*, a UDP-galactose transporter located in the Golgi (36), was unaffected. The fact that the expression of both chaperones increases in the mutant strongly suggests that the absence of AtUTr1 produces a decrease in the influx of UDP-glucose into the ER, reducing the reglucosylation of glycoproteins and perturbing the calnexin/calreticulin cycle, leading to the triggering of the UPR.

The lack of AtUTr1 seems to result in the constitutive activation of the UPR suggesting that these plants are continuously under stress. However, the *AtUTr1* mutant did not show any visible morphological phenotype, even when the plants were grown under conditions such as reducing environment (DTT), darkness, high temperature, high concentration of sucrose, and osmotic stress (data not shown). These results suggest that *Arabidopsis* may have another ER-located NST supplying UDP-glucose into the ER, allowing the plant to cope with the lack of AtUTr1. Alternatively, an NST capable of transporting UDP-glucose may recycle from the Golgi into the ER, as has been shown for a UDP-galactose transporter (45). These possibilities may be alternatives to supply the substrates to UGGT and keep the plants growing apparently normal.

Since AtUTr1 was described as a UDP-galactose/glucose transporter (16), we speculated whether its function is related only to protein folding or whether it is also able to transport UDP-galactose into the ER for galactosylation of glycoconjugates *in vivo*. However, as far as we are aware, no other glucosylation or galactosylation reactions take place in the ER in *Arabidopsis*. Therefore, despite its ability to transport UDP-galactose, we believe that the main role of AtUTr1 in plants is to provide UDP-glucose for UGGT.

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