The PDR5 gene from yeast encodes an ABC (ATP-binding cassette) transporter involved in the ATP-dependent efflux of a variety of structurally unrelated cytotoxic compounds. We report here on the cDNA cloning and characterization of a PDR5 homolog (TUR2) from a higher eukaryote, the aquatic plant Spirodela polyrhiza. We show that TUR2 transcripts accumulate throughout the plant following treatment with the steroid-like hormone, abscisic acid, and that this induction can be repressed by the adenine-derived hormone, kinetin. Furthermore, TUR2 gene expression is induced by environmental stress treatments such as low temperature and high salt. These data indicate that PDR5 homologs are present in plants, that they may function during stress conditions in an analogous fashion to that described in yeast, and that the expression of such ABC transporters is subject to a complex hormonal and environmental regulation.

ATP-binding cassette (ABC) transporters have been implicated in the active movement of a variety of substrates across cellular membranes in a wide variety of organisms, both prokaryotic and eukaryotic (reviewed in Refs. 1 and 2). They thus seem to represent a highly conserved molecular mechanism for the directed transport of specific molecules against a concentration gradient. In addition, they have also become a center of interest for clinical reasons due, first, to their role in the development of multidrug resistance (MDR) (3), and, second, from the finding that the mutated CFTR gene responsible for cystic fibrosis encodes an ABC transporter (4).

Typical ABC transporters include two nucleotide-binding (ABC) domains and two hydrophobic domains containing transmembrane-spanning α-helices (TMS); these domains can reside in one to four proteins (1). Many eukaryotic ABC transporters include all four domains in one protein, and these can be sub-classified into those that have a (TMS6-ABC)2 configuration. The reverse form of four domain transporters have as yet only been found in various yeasts, although “half-size” proteins (ABC-TMS6), such as the white and brown pigment precursor importers, are known in invertebrates (7, 8).

In yeasts, the best characterized reverse form ABC transporter is that encoded by the PDR5 (STS1, YDR1, LEM1) gene (9-12), overexpression of which leads to resistance to a number of structurally unrelated drugs such as cycloheximide, sulfomethuron methyl, sporodesmin, compactin, and cerulenin. Deletion of the gene leads to hypersensitivity to these drugs, as well as to dexamethasone, chloramphenicol, staurosporine, fluphenazine, and mycotoxins. PDR5, therefore, is presumed to function as an efflux pump for cytotoxic compounds in much the same way as the P-glycoprotein MDR1, despite their differing structural organization. Expression of the PDR5 gene has been shown to be under the control of a number of transcriptional regulators (e.g. PDR1, PDR3), suggesting a complex but precise control of expression at the transcriptional level (13). In addition, PDR5 gene expression seems to be under hormonal control, α-factor repressing PDR5 transcript accumulation (10). It has been proposed that the PDR5 gene product plays a physiological role in the externalization of cytotoxic compounds which accumulate during growth, especially in response to environmental stress (11), and that PDR5-like proteins might modulate the levels of steroid hormones in higher eukaryotes (12).

In this paper, we report on the cloning and characterization of a plant cDNA, TUR2, encoding a homolog of the yeast PDR5 ABC transporter. We show that the transcript level is under hormonal control, TUR2 expression being elevated by the steroid-like plant hormone, abscisic acid (ABA), this elevation being reversed by the adenine-derived hormone, kinetin. In addition, environmental stress conditions such as low temperature lead to an elevated level of the TUR2 transcript. These hormonal and environmental effects on TUR2 gene expression are paralleled at the developmental level by the altered fate of new buds produced by the plant (Spirodela polyrhiza), which under conditions of elevated ABA level and cold treatment forms dormant buds, termed turions (14, 15).

The results show that ABC transporters of the PDR5 family are present in plants, and we discuss the significance of the hormonal and environmental stress regulation of expression of this gene with respect to plant growth.

**EXPERIMENTAL PROCEDURES**

**Plant Material**—S. polyrrhiza L. was grown aseptically on 100 ml of half-strength Hutner's medium in 250-ml Erlenmeyer flasks as described previously (14). Each experimental flask was inoculated with one plantlet consisting of between five and eight fronds and allowed to multiply for 7 days before the start of the experimental manipulations. Each experimental sample consisted at this time of between 50 and 100 plants. (RS)−(+)−cis-Trans-Abscisic acid (ABA) was introduced to the
flask as small volumes of concentrated filter-sterilized stock solutions in medium to give final concentrations of between 50 nM and 10 μM. Control cultures were treated with an equal volume of fresh medium. Kinetin was added to the medium as a filter-sterilized 1 mM solution dissolved in 10 mM NaOH to give a final concentration of 20 μM. Control cultures were treated with an equal volume of 10 mM NaOH. When kinetin and ABA were both added to a culture, they were added simultaneously. Cycloheximide, 2,4-D, and NaCl were added to the medium as filter-sterilized stock solutions. The growth of cultures was measured by determining the fresh weight growth constant, k, as described previously (16). The assay for RNA and DNA isolation was frozen in liquid nitrogen before use.

Construction of S. polyrrhiza cDNA Library and Differential Screening—A cDNA library in λ ZAP II (Stratagene) was constructed from poly(A) RNA extracted from fronds of S. polyrrhiza treated with 250 nM ABA for 2 h as previously reported (16). A portion of the unamplified library on nylon filters was differentially screened for ABA-up-regulated cDNA sequences by hybridization to single-stranded 32P-labeled cDNA probes prepared from poly(A) RNA isolated from control fronds (ABA−) and fronds treated for 2 h with 250 nM ABA (ABA+). Filters were washed at high stringency as described previously (16). Plaques that showed a significantly greater signal with the ABA− probe than with the ABA+ probe were considered to represent ABA up-regulated cDNA clones. ABA-up-regulated cDNA clones were plaque-purified by another round of differential screening and recovered in pBluescript SK− by in vivo excision (Stratagene). A full-length cDNA for one of the clones, which we named TUR2, was isolated by rescreening the original cDNA library using a labeled EcoRI/PstI fragment corresponding to the middle 3′-third of the longest cDNA clone as probe.

DNA Sequencing and Sequence Analysis—A deletion library from both ends of the TUR2 cDNA insert was generated by exonuclease III digestion using a nested deletion kit according to the manufacturer’s instructions (Pharmacia). In this way, both strands of the insert cDNA of TUR2 were sequenced by dideoxynucleotide chain termination using TT DNA polymerase (Pharmacia). In some cases Sequenase and dTTP (U. S. Biochemical Corp.) were used to interpret compressions. Remaining gaps were sequenced using synthetic digoxigenin primers.

Searches of GenBank/EBI and SWISS-PROT at the nucleotide and amino acid level for sequences similar to TUR2 were done using the FASTA and TFASTA programs of the UWCGC sequencing package (17). The multiple alignment of the amino acid sequence of TUR2 to the other ABC transporters was done using the Clustal program, whereas individual optimal alignments were done with the GAP program. The prediction of transmembrane regions and an analysis of the topology of the TUR2 protein was done with the TopPred program (18, 19), using the scale of Kyte and Doolittle (20) with an upper cutoff certainty score of 1.35.

Genomic Southern Blot Analysis—S. polyrrhiza genomic DNA was prepared from fronds as described by Rogers and Bendich (21). The DNA was digested with restriction endonucleases, subjected to electrophoresis in 1.1% (w/v) agarose in 0.5× TBE (1× TBE is 89 mM Tris hydrochloride, 2 mM EDTA, pH 8.3) and blotted onto Hybond N membranes (Amersham Corp.) according to the manufacturer’s protocols. The blot was probed with a labeled EcoRI fragment of the TUR2 cDNA insert labeled with digoxigenin using the DIG DNA labeling kit (Boehringer Mannheim). Hybridization, subsequent high-stringency washes (0.1× SSC (1× SSC is 150 mM NaCl, 15 mM trisodium citrate, pH 7.0); 0.1% (w/v) SDS at 65°C) and digoxigenin detection with AMPPD were performed according to the manufacturer’s protocols. Remaining gaps were sequenced using synthetic primers.

RNA Isolation and Northern Blot Analysis—Total RNA from fronds of S. polyrrhiza was prepared by a double guanidine salt method (22) or by the method of Logemann et al. (23), size-fractionated on 1.1% (w/v) agarose-formaldehyde gels, transferred to Hybond N membranes (Amersham), and fixed by baking. The blots were stained with methylene blue and destained before hybridization (24) to detect the amount of RNA loaded onto each lane and to visualize the RNA molecular weight markers (Life Technologies, Inc.) loaded onto an adjacent lane. A 1.4-kbp EcoRI fragment of the TUR2 cDNA insert, purified by electrodialysis from an agarose gel and labeled with α-32PdCTP by the random-priming method (Pharmacia), was hybridized to the RNA blots at 65°C according to the filter manufacturer’s instructions (Amersham). The blots were washed to a final stringency of 0.1× SSC (1× SSC is 180 mM NaCl, 10 mM NaH₂PO₄, 1 mM EDTA, pH 7.7), 0.1% (w/v) SDS at 65°C to ensure specific hybridization and exposed to Hyperfilm MP (Amersham) at −80°C with an intensifying screen for 1–10 days depending on the signal. Hybridization of probes to RNA on Northern blots was quantified by densitometry using a Bio-Rad video densitometer in the transmission mode.

In situ Hybridization Studies and Light Microscopy—In situ hybridizations were carried out as described by Fiersing (25). Tissues were fixed in 4% (w/v) paraformaldehyde in 25% (w/v) glutaraldehyde in paraffin, then sections (7 μm) cut before mounting on poly-L-lysine-coated slides. After prehybridization treatments with proteinase K and acetic anhydride, sections were hybridized with [32P]UTP-labeled RNA probes synthesized from the appropriate promoter (T7 or T3) in the linearized pBluescript SK− clone containing TUR2 using the Stratagene RNA transfection kit (Stratagene). Before hybridization the probes were partially hydrolyzed to a mean length of 150–300 nucleotides. The washing procedure included an RNase A treatment, with a final washing stringency of 2× SSC at room temperature. Slides were coated with Kodak NTB2 emulsion and then exposed at 4°C for 3 weeks.

RESULTS AND DISCUSSION

Isolation and Analysis of the TUR2 cDNA, Which Encodes a Plant ABC Transporter—TUR2 was isolated during the differential screening of a cDNA library from S. polyrrhiza fronds treated for 2 h with 250 nM ABA (the experimentally determined optimum for turion formation). Of the 40 clones representing ABA-up-regulated sequences isolated after two rounds of differential screening, 10 of them were shown by cross-hybridization experiments to represent the same sequence, which was designated TUR2; the longest of the TUR2 cDNA clones (pCS10.4.2) was chosen for further analysis.

After sequencing both strands of the 4.2-kbp cDNA insert of pCS10.4.2 and searching the data bases for similar sequences, it became apparent that TUR2 was very similar to the yeast PDR5 family of ABC transporters. By comparison with the yeast sequences, we knew that the translation initiation site of TUR2 was missing in pCS10.4.2. We therefore rescreened the original cDNA library using a labeled EcoRI/PstI fragment corresponding to the most 3′ 347 bp of this cDNA insert as probe. We obtained several positive clones, and the longest of these, pCS3.94,13, was confirmed by sequencing to be identical to pCS10.4.2, except that it was 368 bp longer at the 5′ end and 13 bp longer at the 3′ end.

The sequence of the 4646-bp TUR2 cDNA is shown in Fig. 1. It encodes an open reading frame of 4323 bp with the most upstream ATG codon beginning at nucleotide 156, and the first in-frame stop codon at nucleotide 4479. The sequence surrounding this initiation codon exactly matches the consensus sequence for initiation in eukaryotes (26, 27). Preceding the translation initiation codon is an in-frame stop codon at position 141, and further upstream are two other stop codons (one in each of the remaining frames).

The protein encoded by TUR2 is 1441 amino acids long and has a predicted molecular weight of 162,696 and a calculated isoelectric point of 8.1. The TUR2-predicted polypeptide displays a structure and domain organization typical of membrane proteins of the ABC transporter superfamily. It is composed of two homologous halves, each comprising an N-terminal hydrophilic domain and a C-terminal hydrophobic domain. According to the algorithm of Kyte and Doolittle (20), six transmembrane-spanning α-helices (TMS) are predicted for each of the two hydrophobic domains of TUR2, resulting in 12 transmembrane segments in the molecule. Each hydrophilic domain located near the N-terminal and central regions of the molecule contains consensus sequences for binding and hydrolysis of ATP typical of the ATP-binding cassette (ABC) domain. TUR2, therefore, is a member of the “full-size” four-domain (ABC-TMS₆) type of ABC transporter. A topological model for the orientation of the TUR2 protein in the membrane is proposed in Fig. 2.

The two ATP-binding cassettes of TUR2 are similar to those conserved throughout the ABC superfamily of transport proteins, consisting of a domain of about 200 amino acids and
comprising the ATP-binding motifs A and B of Walker et al. (28) and, just preceding the Walker B motif, a conserved sequence termed the ABC signature (29) thought to be important in coupling the nucleotide sensor with the transport modules (30). In the amino acid sequence, predicted transmembrane-spanning α-helices according to the Kyte and Doolittle (20) algorithm are underlined, and Walker’s A and B and the ABC signature motifs are double underlined. The use of bold letters is reserved for amino acids, which exactly match the Walker A and B and ABC signature consensus sequences. Consensus sites of N-glycosylation (*) and phosphorylation by tyrosine kinase (Δ), cAMP- and cGMP-dependent protein kinase (●), and protein kinase C (●) are also indicated.

**Fig. 1.** Complete nucleotide sequence of the *S. polyrhiza* TUR2 cDNA and deduced amino acid sequence of the encoded product. Nucleotides are numbered in the 5’ to 3’ direction beginning with the first nucleotide in the cDNA insert (pCS3.94.13). The amino acid sequence (one-letter code) is shown below the nucleotide sequence, and the amino acids are numbered from the initial methionine. In the amino acid sequence, predicted transmembrane-spanning α-helices according to the Kyte and Doolittle (20) algorithm are underlined, and Walker’s A and B and the ABC signature motifs are double underlined. The use of bold letters is reserved for amino acids, which exactly match the Walker A and B and ABC signature consensus sequences. Consensus sites of N-glycosylation (*) and phosphorylation by tyrosine kinase (Δ), cAMP- and cGMP-dependent protein kinase (●), and protein kinase C (●) are also indicated.
A Plant PDR5-like ABC Transporter cDNA

The possession of the degenerate C-terminal ABC signature in TUR2 is a feature seen in the yeast homologs, where the normally conserved glycine in position 4 of the ABC signature is replaced by a glutamate (Glu-998). Interestingly, a similar substitution by a negatively charged residue is seen in the second most common cystic fibrosis mutation, where the same conserved glycine (but in the N-terminal ABC signature) is replaced by an aspartate residue (35). The same mutation in MDR1 (G534D) greatly reduces its expression and MDR activity (30) and in STE6 (G506D) results in a greatly lowered mating efficiency (36). This may indicate that the two ABC domains in "full-size" ABC transporters are not functionally equivalent.

Despite the overall similarity of TUR2 to the yeast proteins, there are notable differences in the N-terminal ABC domain. Whereas the yeast PDR5 subfamily is characterized by degenerate Walker A and B motifs, those of TUR2 are perfect. TUR2, whereas the yeast PDR5 subfamily is characterized by degenerate Walker A and B motifs, those of TUR2 are perfect. TUR2, but does not overlap with the seven upstream ESTs (At13884, At3797, At5784, At6468, and At6687) with significant homology to part of the C-terminal transmembrane domain of TUR2 (ranging from 60 to 43% identity to TUR2), which probably represent the same cDNA and may represent an Arabidopsis TUR2 homolog. There are two additional ESTs (At3366 and At1694) with 46 and 42% identity, respectively, to the same domain of TUR2, which appear to represent two different but related cDNAs. Another EST (At3798) has significant DNA sequence homology to the extreme C terminus of TUR2, but does not overlap with the seven upstream ESTs mentioned above.

Taking these data together, it seems likely that ABC transporters of the PDR5 family are present throughout the higher plant kingdom and that each species probably contains one or a small number of TUR2-like genes. These data fit with the current concept of the ABC transporter as a highly conserved molecular mechanism for the directed transmembrane movement of selected metabolites.

Hormonal and Environmental Regulation of TUR2 Transcript Levels—The expression of many genes of the ABC transporter superfamily have been shown to be under hormonal, chemical, and environmental control (3, 5). The TUR2 cDNA was isolated via a method designed to isolate genes induced by the plant hormone ABA, and, as expected, the TUR2 transcript was found to be up-regulated by ABA. The TUR2 cDNA probe hybridized to an mRNA of ~5.6 kilobases (Fig. 5), which is somewhat larger than the cloned cDNA of 4646 kbp, indicating that the mRNA has a long untranslated region. Fig. 5A reveals that TUR2 is expressed at a very low level in control tissue and that detectable induction of the TUR2 transcript is observable within 30 min of the ABA treatment. A maximum level is achieved after 2 h, representing a 7-fold induction relative to the control level. The rapid rise in TUR2 transcript does not reflect any gross change in the amount of RNA on the blot as quantified by RNA staining (results not shown) and thus indicates a rapid and specific induction of TUR2 by ABA. In a longer term experiment designed to follow the whole period of ABA-induced turion formation, it was found that the TUR2 transcript level remains elevated for a few hours and then declines slowly, reaching control levels 3–4 days after ABA addition (Fig. 5B).

Since turion formation can also be induced by low temperatures (although more slowly than with ABA), we investigated whether the TUR2 transcript accumulated in plants upon cold treatment. During cold-induced turion formation, the level of the TUR2 mRNA increases significantly after 5 days at 15°C and remains at an elevated level thereafter (Fig. 5C). The induction of the TUR2 transcript by low temperature is therefore of a lesser magnitude, slower, and more prolonged than that observed with ABA. The temporal expression of TUR2 thus correlates with the timing of turion induction by ABA and low temperature (16).

The induction of the TUR2 transcript was very sensitive to ABA. It was found that only 50 nM ABA was necessary to

and 34), in decreasing order of homology. A multiple sequence alignment of TUR2 to PDR5, CDR1, and SNO2 is shown in Fig. 3. The similarity of TUR2 to the yeast proteins is conserved along the entire length of the proteins. The possession of the degenerate C-terminal ABC signature in TUR2 is a feature seen in the yeast homologs, where the normally conserved glycine in position 4 of the ABC signature is replaced by a glutamate (Glu-998). Interestingly, a similar substitution by a negatively charged residue is seen in the second most common cystic fibrosis mutation, where the same conserved glycine (but in the N-terminal ABC signature) is replaced by an aspartate residue (35). The same mutation in MDR1 (G534D) greatly reduces its expression and MDR activity (30) and in STE6 (G506D) results in a greatly lowered mating efficiency (36). This may indicate that the two ABC domains in "full-size" ABC transporters are not functionally equivalent.

Despite the overall similarity of TUR2 to the yeast proteins, there are notable differences in the N-terminal ABC domain. Whereas the yeast PDR5 subfamily is characterized by degenerate Walker A and B motifs, those of TUR2 are perfect. TUR2, however, has an imperfectly conserved ABC signature in this region, not reflected in the yeast homologs. TUR2 also contains an extra stretch of 25 amino acids between the Walker A motif and the ABC signature, which is not seen in the same domain of the yeast PDR5 homologs, nor indeed in the C-terminal ABC domain of TUR2 or in either domain of other ABC transporters.

Other ABC transporters displaying significant homology to TUR2 are the "half-size" ABC-TMS6 configuration white (50.4% similarity; 26.0% identity; Ref. 7) and brown (47.8%; 23.0%; Ref. 8) ABC transporters from Drosophila (thought to be importers for the eye pigment precursors guanine and tryptophan; Ref. 37), and the S. cerevisiae permeases YIB3 (51.5% identity; Ref. 38). Homology to other ABC transporters of the TMS6-ABC configuration is mostly confined to the ABC domains, as is the homology to the only other reported example of a plant ABC transporter (the MDR1-like PGP1 from Arabidopsis; Ref. 39).

PDR5 Homologs Are Present Throughout the Plant Kingdom—The result of a genomic Southern hybridization using a 1.4-kbp labeled EcoRI fragment of the TUR2 cDNA as probe is shown in Fig. 4. The hybridization pattern obtained with XbaI and Kpnl is consistent with TUR2 being a single copy gene, although an additional very faint band can be seen with EcoRI and CiaI-restricted DNA. Incomplete DNA restriction or the presence of unknown restriction sites within introns could explain the presence of this larger band, although the existence of a weakly related second gene in Spirodela cannot be ruled out.

A search of the EBI data base revealed significant homology of the TUR2 sequence to ESTs reported as part of the genome sequencing projects for Arabidopsis and rice. We have identified the rice ESTs Os111731 (accession no. D22472) and Os0879a (accession no. D15584) as putative TUR2 homologs with 85% and 66% identity to TUR2, respectively. In the case of Arabidopsis, there are five short partially overlapping ESTs (At13884, At3797, At5784, At6468, and At6687) with significant homology to part of the C-terminal transmembrane domain of TUR2 (ranging from 60 to 43% identity to TUR2), which probably represent the same cDNA and may represent an Arabidopsis TUR2 homolog. There are two additional ESTs (At3366 and At1694) with 46 and 42% identity, respectively, to the same domain of TUR2, which appear to represent two different but related cDNAs. Another EST (At3798) has significant DNA sequence homology to the extreme C terminus of TUR2, but does not overlap with the seven upstream ESTs mentioned above.

Taking these data together, it seems likely that ABC transporters of the PDR5 family are present throughout the higher plant kingdom and that each species probably contains one or a small number of TUR2-like genes. These data fit with the current concept of the ABC transporter as a highly conserved molecular mechanism for the directed transmembrane movement of selected metabolites.

Hormonal and Environmental Regulation of TUR2 Transcript Levels—The expression of many genes of the ABC transporter superfamily have been shown to be under hormonal, chemical, and environmental control (3, 5). The TUR2 cDNA was isolated via a method designed to isolate genes induced by the plant hormone ABA, and, as expected, the TUR2 transcript was found to be up-regulated by ABA. The TUR2 cDNA probe hybridized to an mRNA of ~5.6 kilobases (Fig. 5), which is somewhat larger than the cloned cDNA of 4646 kbp, indicating that the mRNA has a long untranslated region. Fig. 5A reveals that TUR2 is expressed at a very low level in control tissue and that detectable induction of the TUR2 transcript is observable within 30 min of the ABA treatment. A maximum level is achieved after 2 h, representing a 7-fold induction relative to the control level. The rapid rise in TUR2 transcript does not reflect any gross change in the amount of RNA on the blot as quantified by RNA staining (results not shown) and thus indicates a rapid and specific induction of TUR2 by ABA. In a longer term experiment designed to follow the whole period of ABA-induced turion formation, it was found that the TUR2 transcript level remains elevated for a few hours and then declines slowly, reaching control levels 3–4 days after ABA addition (Fig. 5B).

Since turion formation can also be induced by low temperatures (although more slowly than with ABA), we investigated whether the TUR2 transcript accumulated in plants upon cold treatment. During cold-induced turion formation, the level of the TUR2 mRNA increases significantly after 5 days at 15°C and remains at an elevated level thereafter (Fig. 5C). The induction of the TUR2 transcript by low temperature is therefore of a lesser magnitude, slower, and more prolonged than that observed with ABA. The temporal expression of TUR2 thus correlates with the timing of turion induction by ABA and low temperature (16).

The induction of the TUR2 transcript was very sensitive to ABA. It was found that only 50 nM ABA was necessary to
significantly induce the transcript after 2 h. The transcript level increased with increasing ABA concentration until, at over 500 nM ABA, the response was saturated (Fig. 5D). These data are comparable with those for ABA on turion induction, which indicate a threshold concentration of 100–250 nM and a maximal efficacy at 250–500 nM (40).

TUR2 Expression Is Associated with Growth Retardation—Since cytokinins antagonize the effect of ABA on turion formation (41), we tested whether the induction of the TUR2 transcript was similarly affected. Plants were grown with or without the addition of 250 nM ABA in the presence or absence of 20 mM kinetin. While the addition of kinetin along with ABA had no effect on the early (2 h) induction of the TUR2 transcript (Fig. 6A), at later times kinetin progressively attenuated the ABA-induced level of the TUR2 transcript. These results indicated a correlation between TUR2 expression and turion formation.

To further test this correlation, we investigated the effect on TUR2 transcript level of factors unrelated to the induction of turion formation, some of which have been reported to affect the expression of ABC transporters. For example, the expres-
rated by denaturing gel electrophoresis, blotted, and hybridized with a 32P-labeled 1.4-kbp EcoRI fragment of the TUR2 cDNA as probe. The TUR2 transcript size was estimated to be approximately 5.6 kilobases by comparison with RNA size markers. A, RNA (10 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for short periods up to 3 h. The probe was used at 1.5 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 1 day. B, RNA (5 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for up to 7 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. C, RNA (5 μg) from fronds transferred to the turion-inducing temperature of 15°C for different periods up to 14 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. D, RNA (9 μg) extracted from fronds after 2 h cultivation in different concentrations of ABA. The probe was used at 1.1 × 107 dpm ml⁻¹, and the autoradiograph was exposed for 1 day.

Northern blot analysis of the effect of other plant hormones and stress chemicals on the TUR2 transcript level. Total RNA samples were separated by denaturing gel electrophoresis, blotted, and hybridized with a 32P-labeled 1.4-kbp EcoRI fragment of the TUR2 cDNA as probe. The TUR2 transcript size was estimated to be approximately 5.6 kilobases by comparison with RNA size markers. A, RNA (10 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for short periods up to 3 h. The probe was used at 1.5 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 1 day. B, RNA (5 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for up to 7 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. C, RNA (5 μg) from fronds transferred to the turion-inducing temperature of 15°C for different periods up to 14 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. D, RNA (9 μg) extracted from fronds after 2 h cultivation in different concentrations of ABA. The probe was used at 1.1 × 107 dpm ml⁻¹, and the autoradiograph was exposed for 1 day.

Northern blot analysis of the induction of the transcript for TUR2 by ABA and cold. Total RNA samples were separated by denaturing gel electrophoresis, blotted, and hybridized with a 32P-labeled 1.4-kbp EcoRI fragment of the TUR2 cDNA as probe. The TUR2 transcript size was estimated to be approximately 5.6 kilobases by comparison with RNA size markers. A, RNA (10 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for short periods up to 3 h. The probe was used at 1.5 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 1 day. B, RNA (5 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for up to 7 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. C, RNA (5 μg) from fronds transferred to the turion-inducing temperature of 15°C for different periods up to 14 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. D, RNA (9 μg) extracted from fronds after 2 h cultivation in different concentrations of ABA. The probe was used at 1.1 × 107 dpm ml⁻¹, and the autoradiograph was exposed for 1 day.

Northern blot analysis of the effect of other plant hormones and stress chemicals on the TUR2 transcript level. Total RNA samples were separated by denaturing gel electrophoresis, blotted, and hybridized with a 32P-labeled 1.4-kbp EcoRI fragment of the TUR2 cDNA as probe. The TUR2 transcript size was estimated to be approximately 5.6 kilobases by comparison with RNA size markers. A, RNA (10 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for short periods up to 3 h. The probe was used at 1.5 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 1 day. B, RNA (5 μg) from fronds treated with a turion-inducing concentration of ABA (250 nM) for up to 7 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. C, RNA (5 μg) from fronds transferred to the turion-inducing temperature of 15°C for different periods up to 14 days to include the whole period of turion induction. The probe was used at 2.9 × 106 dpm ml⁻¹, and the autoradiograph was exposed for 10 days. D, RNA (9 μg) extracted from fronds after 2 h cultivation in different concentrations of ABA. The probe was used at 1.1 × 107 dpm ml⁻¹, and the autoradiograph was exposed for 1 day.
rions can be viewed as being associated with the decrease in growth that accompanies this process (14) rather than a causal event. This interpretation is strengthened by the analysis of the distribution of ABA-induced TUR2 transcripts by in situ hybridization (Fig. 7). This shows an accumulation of TUR2 mRNA in all parts of the plant, not just those involved in turion formation (14). The TUR2-encoded ABC transporter is thus likely to be active in all cells of the plant.

Levels of the PDR5 transcript in yeast (to which TUR2 is homologous) have been shown to be sensitive to the presence of the α-mating factor (10), and it has been suggested that PDR5 expression is linked to environmental stress, specifically in the excretion of cytotoxic metabolites (11). The observation that the TUR2 transcript level is increased following treatment with ABA (a “stress” hormone), cold, and salt stress, raises the possibility that the PDR5-like genes in plants represent ABC transporters also involved in the movement of metabolites which accumulate after stress treatment. What these metabolites might be is as yet unknown.

It has recently been suggested that PDR5-like transporters might play a role in affecting the balance of steroid hormones in higher eukaryotes (12), and the expression of other ABC transporters has been shown to be up-regulated by steroid hormones (5). The observation that in plants a hormone structurally related to steroids affects the expression of a gene encoding an ABC transporter is thus intriguing. Indeed, since it has been shown that ABC transporters are sometimes induced by their own substrates (5, 11, 42), we performed transient transformation assays in tobacco protoplasts to search for a possible effect of overexpression of the TUR2 cDNA on ABA transport. However, so far our results have proved negative (data not shown).

At present, we cannot say in which membrane the TUR2-encoded protein is situated. However, evidence from studies on the PDR5 gene product in yeast suggests a plasma membrane localization (43, 44), and it has been pointed out that “full-size” ABC transporters (such as TUR2) tend to be located in the plasma membrane (5).

In conclusion, the TUR2 cDNA encodes a novel plant ABC transporter with homology to the well characterized yeast PDR5 gene product. The plant PDR5 homolog is subject to a detailed molecular genetic analysis of the homologous gene in Arabidopsis, should enable progress to be made as to the physiological function of this class of ABC transporters in a higher eukaryote.

Acknowledgments—We thank Prof. N. Amrhein for support and encouragement, Dr. Dieter Rubli for photographic work, and Martha Stadler-Waibel for technical assistance.

REFERENCES

1. Higgins, C. F. (1992) Annu. Rev. Cell Biol. 8, 67–113
2. Fath, M. J., and Kolter, R. (1993) Microbiol. Rev. 57, 995–1017
3. Endicott, J. A., and Ling, V. (1989) Annu. Rev. Biochem. 58, 137–171
4. Riordan, J. R., Rommens, J. M., Kerem, B.-S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chau, J.-L., Drumm, M. L., Iannuzzi, M. C., Collins, F. S., and Tsui, L.-C. (1989) Science 245, 1056–1073
5. Gotteeman, M., and Pastan, I. (1993) Annu. Rev. Biochem. 62, 385–427
6. Kuchler, K., Sterne, R. E., and Thörner, J. (1993) EMBO J. 12, 3973–3984
7. O’Hare, K., Murphy, C., Lewis, R., and Rubin, G. M. (1986) J. Mol. Biol. 180, 437–455
8. Dresen, T. D., Johnson, D. H., and Henikoff, S. (1988) Mol. Cell. Biol. 8, 5206–5215
9. Balzi, E., Wang, M., Leterme, S., Van Dyck, L., and Goffeau, A. (1994) J. Biol. Chem. 269, 2206–2214
10. Bissinger, P. H., and Kuchler, K. (1994) J. Biol. Chem. 269, 4180–4186
11. Hirata, D., Yano, K., Miyahara, K., and Miyakawa, T. (1994) Curr. Genet. 26, 285–294
12. Krali, A., Bohen, S. P., and Yamamoto, K. R. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 4701–4705
13. Balzi, E., and Goffeau, A. (1994) Biochim. Biophys. Acta 1187, 152–162
14. Smart, C. C., and Trewavas, A. J. (1987) Plant Cell Environ. 6, 507–514
15. Smart, C., Longland, J., and Trewavas, A. (1987) in Molecular Biology of Plant Growth Control (Fox, J. E., and Jacobs, M., eds) pp. 345–359, Alan R. Liss, New York.
16. Smart, C. C., and Fleming, A. J. (1993) Plant J. 4, 279–293
17. Devereaux, J., Haeberli, P., and Smithies, O. (1984) Nucleic Acids Res. 12, 387–395
18. von Heijne, G. (1992) J. Mol. Biol. 225, 487–494
19. Sipos, L., and von Heijne, G. (1993) Eur. J. Biochem. 213, 1333–1340
20. Kyte, J., and Dodgshie, R. F. (1982) J. Mol. Biol. 157, 105–132
21. Rogers, S. O., and Bendich, A. J. (1988) in Plant Molecular Biology Manual (Gelvin, S. B., and Schilperoort, R. A., eds) Part A6, pp. 1–10, Kluwer Academic Publishers, Dordrecht, The Netherlands
22. Han, J.-H., Stroutawa, C., and Rutter, W. J. (1987) Biotechnology 6, 1617–1625
23. Logemann, J., Schell, J., and Willmitzer, L. (1987) Anal. Biochem. 163, 16–20
24. Herrin, D. L., and Schmidt, G. W. (1988) BioTechniques 6, 196–200
25. Fleming, A. J. (1990) in Gene Transfer to Plants (Potyvaks, I., and Spanenberg, G., eds) pp. 273–285, Springer Verlag, Heidelberg
26. Cavener, D. R., and Ray, S. C. (1991) Nucleic Acids Res. 19, 3185–3192
27. Kosak, M. (1991) J. Biol. Chem. 266, 19867–19870
28. Walker, J. E., Saraste, M., Runswick, M. J., and Guy N. J. (1982) EMBO J. 1, 945–951
29. Shyamala, V., Bichwals, V., Beal, E., and Ames, B. G. L. (1991) J. Biol. Chem. 266, 18714–18719
30. Hof, T., Denner, A., Hadam, M. R., Riordan, J. R., and Tümmler, B. (1994) J. Biol. Chem. 269, 20575–20583
31. Prasad, R., Weichert, F., Goffeau, A., and Balzi, E. (1995) Curr. Genet. 27, 320–329
32. Sorensen, J., Haase, E., and Brendel, M. (1993) Mol. & Gen. Genet. 236, 214–218
33. Nagao, K., Taguchi, Y., Arikawa, M., Kadokura, H., Takatsuki, A. Yoda, K., and Yamashita, M. (1995) J. Bacteriol. 177, 1536–1543
34. Turi, T. G., and Rose, J. K. (1995) Biochim. Biophys. Res. Commun. 213, 410–418
35. Cutting, R. G., Kasch, L. E., Rosenberg, B. J., Trewavas, A., and Neyfakh, A. (1994) Plant Physiol. 105, 623–632
36. Berkower, C., and Michaelis, S. (1991) EMBO J. 10, 3777–3785
37. Ewart, G. D., Cannell, D., Cox, G. B., Howells, A. J. (1994) J. Biol. Chem. 269, 10370–10377
38. Purnelle, B., Skala, J., and Goffeau, A. (1991) Yeast 7, 867–872
39. Dudler, R., and Hertig, C. (1992) J. Biol. Chem. 267, 5882–5888
40. Smart, C. C., Fleming, A. J., Chaloupková, K., and Hanke, D. E. (1995) Plant Physiol. 106, 320–329
41. Chaloupková, K., and Smart, C. C. (1994) Plant Physiol. 105, 497–507
42. Ahmed, M., Borsch, C. M., Taylor, S. S., Vásquez-Laslop, N., and Neyfakh, A. A. (1994) J. Biol. Chem. 269, 28506–28513
43. EGner, R., Mahé, Y., Pandit, R., and Kuchler, K. (1995) Mol. Cell. Biol. 15, 5879–5887
44. Deottning, A., Kołaczkowski, M., Balzi, E., and Goffeau, A. (1994) J. Biol. Chem. 269, 12797–12803