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Biochimica et Biophysica Acta 1370 (1998) 187–191

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Short sequence paper

ERD6, a cDNA clone for an early dehydration-induced gene of *Arabidopsis*, encodes a putative sugar transporter [1]

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Received 31 October 1997; revised 8 January 1998; accepted 8 January 1998

Abstract

Previously, we constructed a cDNA library from *Arabidopsis* plants that were exposed to dehydration stress for 1 h and obtained the ERD6 clone. Here we report that the ERD6 cDNA consists of 1741 bp and encodes a polypeptide of 496 amino acids having a predicted molecular weight of 54,354. The putative polypeptide of ERD6 is related to those of sugar transporters of bacteria, yeasts, plants and mammals. Hydropathy analysis revealed that ERD6 protein has 12 putative transmembrane domains and a central hydrophilic region. Sequences that are conserved at the ends of the 6th and 12th membrane-spanning domains of sugar transporters are also present in ERD6. These data suggest that ERD6 encodes a sugar transporter. Genomic Southern blots indicate that the ERD6 gene is a member of a multigene family in the *Arabidopsis* genome. The expression of the ERD6 gene was induced not only by dehydration but also by cold treatment. © 1998 Elsevier Science B.V.

Keywords: Cold stress; Dehydration; Sugar transporter; (*Arabidopsis thaliana*)

Drought and salinity stresses are major factors that limit growth and productivity of higher plants [1]. Since plants are immobile, they respond to such stresses with physiological, developmental and biochemical changes including the synthesis of a number of proteins [2,3].

We are interested in understanding plant responses to dehydration at the molecular level. To study the signal transduction pathway that links dehydration stress and gene expression, and to investigate the functions of the products for water stress-inducible genes, we have isolated and characterized several cDNAs and genes that were responsive to dehydration or salinity stress [4]. To this end, we obtained cDNA clones for 9 RD (responsive to desiccation) genes [5], a *myb*-homolog [6], 16 ERD (early-responsive to dehydration) genes [7], two Ca^{2+}-dependent protein kinases [8], a soluble epoxide hydrolase [9], a phosphatidylinositol-specific phospholipase C [10], a Δ^1-pyrroline-5-carboxylate synthetase [11], ribosomal-protein S6 kinase homologues [12], a mitogen-activated protein kinase (MAPK), a MAPK kinase kinase (MAPKKK) [13], and so on.

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[1] The nucleotide sequence reported in this paper has been submitted to DDBJ with the accession number of D89051.

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PII S0005-2736(98)00007-8

ERD cDNA clones, isolated using differential screening procedures, correspond to genes that are expressed after dehydration for 1 h in *Arabidopsis thaliana* [7]. Sequence analysis of ERD clones revealed that ERD1 was homologous to a cDNA for the regulatory subunit of the Clp ATP-dependent protease in *Escherichia coli*, ERD5 encoded a mitochondrial proline dehydrogenase, ERD10 and ERD14 were similar to cDNAs for group II late embryogenesis abundant (LEA) proteins, ERD11 and ERD13 were homologous to cDNAs for glutathione *S*-transferases, and ERD2, ERD8, and ERD16 were identical to cDNAs for heat shock proteins HSP70-1, HSP81-2, and the ubiquitin extension protein, respectively [14]. In this paper, we report the characterization of one of the remaining ERD clones, ERD6.

Fig. 1 shows the nucleotide sequence and the deduced amino acid sequence of ERD6. ERD6 cDNA consists of 1741 bp encoding a polypeptide of 496 amino acids having a predicted molecular weight of 54,354. The deduced amino acid sequence of ERD6 was compared to those compiled in databases and was found to be related to sugar transporters in a variety of organisms. As shown in Table 1, amino acid identity was approximately 30%, whereas amino acid similarity was about 70%. Hydropathy analysis revealed that the ERD6 protein possessed 12 putative transmembrane domains and a central hydrophilic region that are common characteristics of sugar transporters (Fig. 2). In addition, we found the sequences of PESPRXL and PETKGXXXE at the ends of the 6th and 12th membrane-spanning domains, respec-

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   1  GATCCGGGATGGGAAAGAAGCAGGAGGAGACTTTTTGGAATGGAGAGACAAAAGAGCATGGAAAAAGGGTTACTCAGGAAGAGCTTAAGCATACGTGAGA  100
                                                M  E  R  Q  K  S  M  E  K  G  L  L  R  K  S  L  S  I  R  E  R

 101  GAAAGTTCCCTAACGAAGACGCTTTCTTAGAATCCGGTTTATCGAGGAAGTCTCCGCGAGAGGTCAAGAAACCTCAAAACGACGATGGTGAATGTCGTGT  200
         K  F  P  N  E  D  A  F  L  E  S  G  L  S  R  K  S  P  R  E  V  K  K  P  Q  N  D  D  G  E  C  R  V

 201  TACCGCCTCTGTTTTCCTCAGCACCTTTGTTGCCGTATCAGGCTCCTTCTGTACCGGTTGTGGCGTTGGTTTTTCATCGGGTGCACAAGCAGGGATTACC  300
         T  A  S  V  F  L  S  T  F  V  A  V  S  G  S  F  C  T  G  C  G  V  G  F  S  S  G  A  Q  A  G  I  T

 301  AAAGATTTATCTCTCTCCGTTGCAGAATACTCAATGTTCGGGTCGATCTTGACATTAGGAGGCTTGATCGGTGCAGTATTCAGCGGTAAAGTCGCTGATG  400
         K  D  L  S  L  S  V  A  E  Y  S  M  F  G  S  I  L  T  L  G  G  L  I  G  A  V  F  S  G  K  V  A  D  V

 401  TCTTGGGAAGAAAACGGACGATGTTGTTTTGCGAATTCTTCTGTATCACAGGCTGGCTTTGTGTAGCATTGGCTCAGAATGCAATGTGGCTGGACTGTGG  500
         L  G  R  K  R  T  M  L  F  C  E  F  F  C  I  T  G  W  L  C  V  A  L  A  Q  N  A  M  W  L  D  C  G

 501  AAGATTGTTACTTGGAATCGGCGTTGGTATATTTAGCTACGTGATTCCGGTGTATATAGCCGAAATTGCACCTAAACATGTCCGAGGATCGTTTGTGTTC  600
         R  L  L  L  G  I  G  V  G  I  F  S  Y  V  I  P  V  Y  I  A  E  I  A  P  K  H  V  R  G  S  F  V  F

 601  GCCAATCAGTTGATGCAAAATTGCGGAATTTCACTCTTCTTCATCATTGGCAATTTTATTCCATGGAGACTACTAACAGTAGTCGGATTGGTGCCATGTG  700
         A  N  Q  L  M  Q  N  C  G  I  S  L  F  F  I  I  G  N  F  I  P  W  R  L  L  T  V  V  G  L  V  P  C  V

 701  TGTTCCACGTCTTTTGTTTATTTTTCATCCCCGAATCTCCAAGATGGCTGGCGAAGTTAGGTCGTGATAAAGAATGCCGATCTTCGTTGCAACGCCTTAG  800
         F  H  V  F  C  L  F  F  I  P  E  S  P  R  W  L  A  K  L  G  R  D  K  E  C  R  S  S  L  Q  R  L  R

 801  GGGATCTGACGTCGATATTTCTCGTGAAGCAAACACAATTCGAGATACCATTGACATGACAGAAAACGGTGGTGAAACTAAGATGTCTGAATTGTTTCAG  900
         G  S  D  V  D  I  S  R  E  A  N  T  I  R  D  T  I  D  M  T  E  N  G  G  E  T  K  M  S  E  L  F  Q

 901  AGACGATACGCATATCCGTTAATTATCGGAGTTGGTTTAATGTTTTTGCAACAATTGTGTGGGAGCTCCGGTGTTACCTATTATGCTAGTAGCCTCTTCA 1000
         R  R  Y  A  Y  P  L  I  I  G  V  G  L  M  F  L  Q  Q  L  C  G  S  S  G  V  T  Y  Y  A  S  S  L  F  N

1001  ACAAAGGAGGATTTCCAAGTGCTATTGGCACATCCGTAATAGCCACAATTATGGTTCCAAAAGCAATGCTGGCAACAGTCCTAGTCGATAAAATGGGGAG 1100
         K  G  G  F  P  S  A  I  G  T  S  V  I  A  T  I  M  V  P  K  A  M  L  A  T  V  L  V  D  K  M  G  R

1101  GAGAACGCTCCTAATGGCTTCTTGTTCTGCAATGGGTTTGAGTGCTTTGCTCTTAAGTGTTTCTTACGGTTTCCAGTCGTTTGGCATTCTTCCAGAACTC 1200
         R  T  L  L  M  A  S  C  S  A  M  G  L  S  A  L  L  L  S  V  S  Y  G  F  Q  S  F  G  I  L  P  E  L

1201  ACTCCCATCTTCACTTGCATCGGCGTCTTGGGTCACATTGTGTCATTTGCCATGGGAATGGGAGGACTACCATGGATTATAATGGCTGAGATATTTCCGA 1300
         T  P  I  F  T  C  I  G  V  L  G  H  I  V  S  F  A  M  G  M  G  G  L  P  W  I  I  M  A  E  I  F  P  M

1301  TGAATGTGAAAGTGTCAGCTGGGACCTTAGTTACTGTAACCAATTGGTTATTTGGTTGGATTATCACATACACTTTCAATTTTATGCTAGAATGGAATGC 1400
         N  V  K  V  S  A  G  T  L  V  T  V  T  N  W  L  F  G  W  I  I  T  Y  T  F  N  F  M  L  E  W  N  A

1401  ATCAGGAATGTTCCTCATCTTCTCAATGGTCTCCGCCAGTTCGATCGTATTTATATACTTTTTGGTACCTGAGACAAAAGGCCGATCACTTGAAGAAATA 1500
         S  G  M  F  L  I  F  S  M  V  S  A  S  S  I  V  F  I  Y  F  L  V  P  E  T  K  G  R  S  L  E  E  I

1501  CAAGCACTGCTCAACAACTCTGTGCAATAATATCATTTTTCTTTTTCTTTTTGGGTAAATGATCATATATATAAGTCGATTGTTGTTATTTGGTGTGAGT 1600
         Q  A  L  L  N  N  S  V  Q  *
1601  TTGAATGTGATCCGTGTGCGTATCAAATTTTGGATGGGAAATTTGAAACAGTAAAAATTTGTATATTCCTCGTTTGGGAAAAAAAAAAAAAAAAAAAAA 1700
1701  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1741
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Fig. 1. Nucleotide and deduced amino acid sequence of the ERD6 cDNA. Nucleotides are numbered from the first base of the cDNA clone. The deduced amino acid sequence is indicated below the nucleotide sequence. An asterisk indicates a termination codon. Conserved amino acid sequences among sugar transporters at the end of the 6th and 12th transmembrane domains are underlined.

Table 1
The extent of the amino acid homology between members of (putative) sugar transporters

Transporters	1	2	3	4	5	6	7	8
(1) ERD6	*	42 [81]	29 [68]	30 [67]	29 [72]	31 [68]	29 [69]	31 [67]
(2) U43629		*	30 [71]	30 [70]	33 [73]	31 [69]	28 [67]	30 [67]
(3) P30605			*	30 [72]	30 [69]	33 [72]	25 [64]	27 [66]
(4) P37021				*	29 [69]	65 [90]	33 [70]	33 [74]
(5) P11168					*	26 [70]	27 [69]	29 [70]
(6) P09830						*	31 [70]	34 [73]
(7) P09098							*	28 [68]
(8) P15686								*

The extent of the identity [similarity] (%) between sequences was calculated using the GENETYX software system. The accession numbers were used to indicate sources: U43629, *Beta vulgaris* integral membrane protein (Chiou and Bush [15]); P30605, yeast *myo*-inositol transporter 1 (Nikawa et al. [16]); P37021, *E. coli* galactose transporter; P11168, human glucose transporter type 2 (Fukumoto et al. [17]); P09830, *E. coli* arabinose transporter (Maiden et al. [18]); P09098, *E. coli* xylose transporter (Davis and Henderson [19]); P15686, *Chlorella* proton/hexose cotransporter (Sauer and Tanner [20]).

tively. These sequences are conserved among the sugar transporter subgroup of the major facilitator superfamily [22,15]. Taken together, these data suggest that ERD6 encodes a sugar transporter.

To investigate ERD6 function in vivo, we expressed ERD6 protein in yeast cells and measured their sugar transporter activities as described by Sauer et al. [22]. No transport was detected when ^{14}C-labeled 3-*O*-methylglucose, D-galactose, D-fructose, or D-xylose were used as a substrate (data not shown). In this regard, it is interesting to note that ERD6 protein is most closely related to a sugar beet putative sugar transporter whose sugar transport activity was also reported to be undetectable in yeast cells [15]. These facts imply that ERD6 protein may transport specific sugar substrate(s) that we have not yet tested, or that the native structure of the ERD6 protein in yeast cells may be different from that in *Arabidopsis*. Another possibility is that the protein may be targeted to an intracellular membrane. Indeed, the putative transporter of sugar beet was a tonoplast membrane protein in plant cells [15].

To estimate the size of the ERD6 gene family, *Arabidopsis* genomic DNA was digested with five

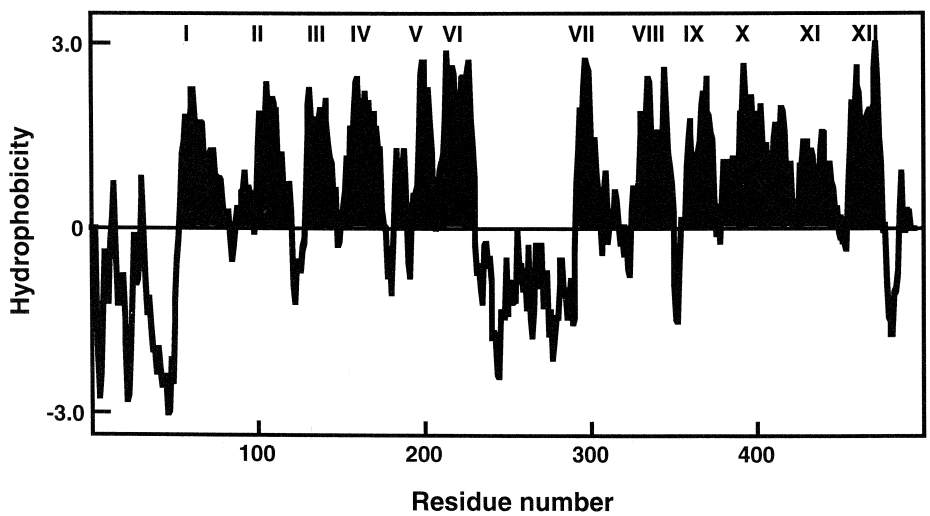

Fig. 2. Hydropathy plot of the ERD6 protein. The Kyte–Doolittle hydropathy profile [21] of the protein was calculated by using a window of eight residues. Roman numerals indicate hydrophobic regions that are hypothesized to be transmembrane domains.

restriction enzymes and hybridized to [^{32}P]-labeled ERD6 cDNA under both high and low stringency conditions (Fig. 3). The ERD6 cDNA has one internal *Eco*RI restriction site and no internal restriction site for *Pst*I, *Xba*I, *Hin*dIII, and *Bam*HI restriction enzymes. When the low-stringency hybridization condition was used, ERD6 cDNA hybridized with many DNA restriction fragments. This suggests that the ERD6 gene belongs to a multigene family in the *Arabidopsis* genome.

The expression of the ERD6 gene in response to dehydration stress was investigated by Northern blot analysis (Fig. 4). These experiments were carried out using high stringency conditions to detect ERD6-specific transcripts. Before dehydration, the level of ERD6 mRNA was very low. ERD6 mRNA concentration was maximal after 1 h of dehydration. At 2 h, the level of ERD6 mRNA had decreased, and by 5 h it had returned to the same low level observed before dehydration. We also found that a different stress, cold treatment, induced ERD6 gene expression within 1 h. The elevated level of the ERD6 mRNA decreased 5 h after the onset of the cold treatment.

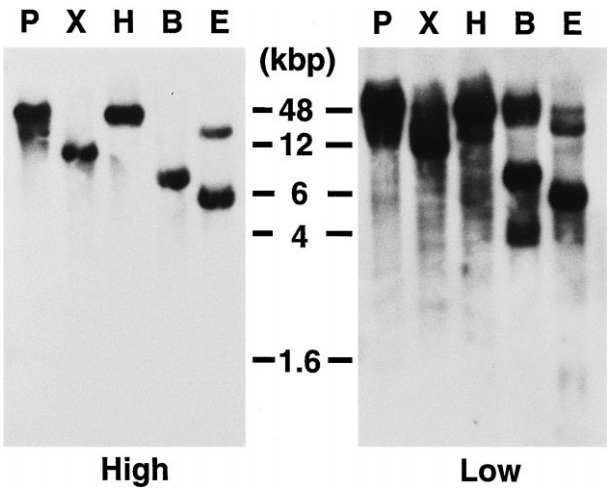

Fig. 3. Southern blot analysis of *Arabidopsis* genomic DNA. Genomic DNA was digested with the indicated restriction endonucleases, fractionated on a 0.7% agarose gel and transferred to a nylon membrane. The membrane was cut into two pieces, hybridized with [^{32}P]-labeled ERD6 cDNA at 42°C and washed in either 0.5× SSC/0.5% SDS at 50°C (low stringency) or 0.1× SSC/0.1% SDS at 65°C (high stringency). 'High' and 'Low' represent high- and low-stringency hybridization conditions, respectively. P, *Pst*I; X, *Xba*I; H, *Hin*dIII; B, *Bam*HI; E, *Eco*RI. The sizes of DNA markers are indicated in kbp.

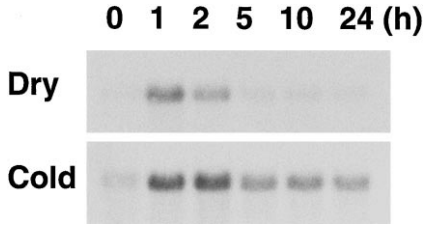

Fig. 4. Northern blot analysis of ERD6 mRNA after dehydration or cold treatment. Ten micrograms of total RNA, extracted from 4-week-old *A. thaliana* plants, were loaded in each lane. Samples were prepared from plants that had been treated by dehydration or incubated at 4°C for the indicated period of time. RNA was fractionated on 1.2% agarose gels that contained formaldehyde and transferred to nylon membranes. Filters were hybridized with a [^{32}P]-labeled fragment of ERD6 cDNA at 42°C and washed in 0.1× SSC/0.1% SDS at 65°C.

However, the level of ERD6 mRNA was still higher in 24 h cold-treated plants than in the nontreated plans. These results show that two different stresses, dehydration and cold, transiently induce ERD6 gene expression.

The physiological function of the ERD6 protein in plants is unknown. However, since ERD6 gene expression was induced in both dehydrated or cold-treated plants, ERD6 protein might function in the redistribution of sugars that are used as energy sources to protect cells from these stresses. In this regard it is interesting to note that the products of some stress-inducible genes seem to require energy in the form of ATP for their functions [7,8,12,13,23,24]. It is also possible that sugar redistribution is needed to directly protect cells against dehydration and/or cold stress. For example, it has been reported that some sugar alcohols act as osmoprotectants in plant cells [25,26].

The authors are grateful to Dr. N. Sauer (Universität Regensburg, Germany) for providing the vectors for expression in yeast cells. The authors wish to thank Dr. R.L. Fischer (University of California, Berkeley) for his critical reading of the manuscript. This work was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences, the Human Frontier Science Program, the Special Coordination Funds of the Science and Technology Agency of the Japanese Government and by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan. T. Kiyosue was supported by a fellowship from the Science and Technology Agency of Japan.

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