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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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 Ca2+−dependent protein kinases [8], a soluble epoxide hydrolase [9], a phosphatidylinositol-specific phospholipase C [10], a Δ1-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.
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 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-

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

<table>
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<tr>
<th>Transporters</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>(1) ERD6</td>
<td>*</td>
<td>42</td>
<td>29</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
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<tr>
<td>(2) U43629</td>
<td>*</td>
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<td>30</td>
<td>33</td>
<td>31</td>
<td>28</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>(3) P30605</td>
<td>*</td>
<td>30</td>
<td>30</td>
<td>33</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>(4) P37021</td>
<td>*</td>
<td>29</td>
<td>65</td>
<td>90</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>34</td>
</tr>
<tr>
<td>(5) P11168</td>
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<td>26</td>
<td>70</td>
<td>31</td>
<td>29</td>
<td>29</td>
<td>29</td>
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<tr>
<td>(6) P09830</td>
<td>*</td>
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<td>(7) P09098</td>
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<td>28</td>
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<tr>
<td>(8) P15686</td>
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</tr>
</tbody>
</table>

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]).

Table 1 details the amino acid homology between members of putative sugar transporters. ERD6 encodes a sugar transporter, and its function was investigated in vivo by expressing ERD6 protein in yeast cells and measuring sugar transporter activities. ERD6 protein is most closely related to sugar beet putative sugar transporter whose activity was undetectable in yeast cells. ERD6 protein may transport specific sugar substrate(s) not yet tested, or 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. To estimate the size of the ERD6 gene family, Arabidopsis genomic DNA was digested with five restriction enzymes.

Fig. 2. Hydropathy plot of the ERD6 protein. The Kyte–Doolittle hydrophobicity 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 EcoRI restriction site and no internal restriction site for PstI, XbaI, HindIII, and BamHI 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.

However, the level of ERD6 mRNA was still higher in 24 h cold-treated plants than in the nontreated plants. 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].

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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 $^{35}$P-labeled fragment of ERD6 cDNA at 42°C and washed in 0.1× SSC/0.1% SDS at 65°C.
References