Plant Physiol. (1993) 102: 689–690

Plant Gene Register

Nucleotide Sequence of an *Arabidopsis thaliana* Turgor-Responsive *TMP-B* cDNA Clone Encoding Transmembrane Protein with a Major Intrinsic Protein Motif[1]

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Water is one of the most important elements in sustaining life. Therefore, shortage of water produces a stress on any living organism. Plants exposed to water stress undergo a series of physiological, biochemical, and molecular changes. The nature of the response varies with the severity and duration of the stress. Some of these responses include closure of stomata, decrease in photosynthesis, increase in ABA level (Zeevart and Creelman, 1988), and alteration in gene expression (Skriver and Mundy, 1990; Bray, 1991). Here we describe a cDNA clone encoding TMP-B from *Arabidopsis thaliana*. The protein encoded by it is a second member of the gene family of the recently cloned *A. thaliana TMP-A* and is very homologous to that encoded by the turgor-responsive 7a cDNA cloned from pea (Guerrero et al., 1990).

Screening of the cDNA library is described elsewhere (Bar-Zvi et al., 1992; Shagan and Bar-Zvi, 1993). Here we describe one of these clones, termed T4. The DNA insert was subcloned to pBluescript and was subjected to restriction and sequence analysis. Sequence analysis revealed that the 2188-bp T4 clone is composed of a 1057-bp cDNA sequence ligated "head to head" to RNP-T cDNA. It is interesting that TMP-A was also isolated as a ligate with RNP-T. We cannot explain this coincidence.

The 1057-bp cDNA contained an open reading frame of 858 bp encoding 286 amino acid residues (Table I). The TMP-B polypeptide, like the TMP-A polypeptide, is shorter by three amino acid residues than that encoded by the turgor-responsive 7a cDNA cloned from pea (Guerrero et al., 1990). The overall amino acid identities of TMP-B with TMP-A and 7a are 93 and 84%, whereas similarities are 96.5 and 91%, respectively. The nucleotide sequence of the coding region of TMP-B showed 82% identity with TMP-A and 74% identity with 7a. Of the 286 codons, 137 were altered between TMP-A and TMP-B. Eighty percent of these changes are in the third position of the codon. The nucleotide change in 118 of the altered codons did not result in a change of the amino acid encoded by it; thus, the two polypeptides differ in only 17 amino acid residues. No significant sequence homology was found in the noncoding sequences. TMP-B, like TMP-A, has two insertions compared to the 7a polypeptide: three amino acids between residues 28 and 29 and one amino acid between residues 155 and 156 of TMP-B. In addition, both of the *Arabidopsis* TMP-A and TMP-B polypeptides have one additional residue in their carboxy terminals compared with the pea polypeptide.

The protein also contains an MIP motif (SGGHINPAVT) (Table I), found first in the major intrinsic protein of the bovine lens fiber membrane (Gorin et al., 1984). This motif is found in many transmembrane proteins of plant and nonplant origin (Yamamoto et al., 1990; Shagan and Bar-Zvi, 1993).

Table I. *Characteristics of A. thaliana TMP-B cDNA*

Organism:
Arabidopsis thaliana (L.) Heynh., Columbia ecotype, Brassicaceae.
Location in Genome:
Nuclear genome.
Strategy of Cloning:
cDNA library in λZAP screened by DNA hybridization.
Sequencing Methods:
Restriction fragment subcloning and homologous oligonucleotides; complete dideoxy sequencing of both strands in pBluescript (Stratagene) using Sequenase (United States Biochemical).
Feature of cDNA:
Contains 1057 nucleotides consisting of 13 nucleotides 5′ untranslated, 858 nucleotides open reading frame, and 186 nucleotides 3′ untranslated; no polyadenylation signal or poly(A) tail were observed at the 3′ end of the cDNA.
Structural Feature of Protein:
Protein contains 286 amino acids; M_r = 30,612. MIP motif: amino acid residues 109 to 118. Six putative transmembrane sequences: amino acid residues 53 to 75, 89 to 111, 132 to 150, 175 to 198, 204 to 227, and 263 to 280.
Subcellular Localization of Protein:
Not determined.

[1] This work was supported by a grant from the Basic Research Foundation of The Israel Academy of Sciences and Humanities to D.B-Z. D.B-Z. is an incumbent of the Judith and Murray Shusterman chair for carrier development in microbiology.

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Abbreviation: MIP, major intrinsic protein.

A structure containing six transmembrane helices is common in ion channel proteins (Maelicke, 1988). Hydropathy plots by the method of Kyte and Doolittle (1982) revealed six stretches of hydrophobic amino acids, which could correspond to membrane-spanning domains (Table I). Most of the potential membrane-spanning helices are bordered by charged residues or by helix-breaking amino acids. Like TMP-A (Shagan and Bar-Zvi, 1993), most of the amino acid residues that vary between the TMP-B and 7a polypeptides are localized in the nonhydrophobic regions. On the other hand, the 18 amino acid residues that differ between *Arabidopsis* TMP-A and TMP-B polypeptides are scattered. Some of the proteins containing the MIP motif were suggested to be transporters (Yamamoto et al., 1990; Shagan and Bar-Zvi, 1993). All of the above suggests that the proteins encoded by members of the *TMP* gene family might be ion transporters, possibly involved in turgor regulation (Guerrero et al., 1990).

ACKNOWLEDGMENT

We wish to thank Professor J. Ecker for the cDNA library.

Received December 15, 1992; accepted December 21, 1992.
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The EMBL accession number for the sequence reported in this article is X69294.

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