Plant Molecular Biology **26**: 2003–2007, 1994.

Short communication

Isolation and expression analysis of two rice genes encoding the major intrinsic protein

Qiang Liu, Masaaki Umeda and Hirofumi Uchimiya*
*Cellular Function Laboratory, Institute of Molecular and Cellular Biosciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan (*author for correspondence)*

Received 29 March 1994; accepted in revised form 20 September 1994

Key words: rice, major intrinsic protein, tonoplast intrinsic protein, channel protein, transmembrane domain

Abstract

We isolated two rice cDNAs (rMip1 and rTip1) which are homologous to the genes encoding the major intrinsic protein (Mip) (soybean nod-26 and *Arabidopsis* γ-Tip), respectively. Expression of rTip1 in shoots and roots of rice seedlings was enhanced by water stress, salt stress and exogenous ABA. rMip1 was expressed only in shoots. Although mRNA level of rMip1 in shoots was induced to a small extent by exogenous ABA, it did not show any increase under water or salt stress over the course of 12 h. On the basis of the differential expression patterns and evolutional distinctions, it is suggested that the possible channel proteins encoded by rMip1 and rTip1 genes may function in different transport systems.

Genes similar to that encoding the major intrinsic protein (Mip) isolated from bovine lens fiber membrane [3] have been found in some dicotyledonous plants, such as soybean [2, 10, 14], bean [6], pea [4], tobacco [19], *Arabidopsis* [5, 7, 18, 19] and *Antirrhinum* [1]. All of the proteins in the Mip family have six putative transmembrane domains and are postulated to form membrane channels for transport of water, ions, solutes or metabolites.

Based on cellular locations of recently published plant Mips, they can be classified into two types, nod-26 and Tips (tonoplast intrinsic proteins). The soybean nod-26 gene is expressed only in infected cells of nodules and its protein is located in the peribacteroid membrane [9, 10]. Thus, this channel protein may play a role in metabolite transport in symbiotic nitrogen fixation [9]. So far, the existence of its homologues (high identity throughout the entire amino acid sequence) has been found only in roots. Tips are a group of intrinsic proteins located in the tonoplast of the seeds of bean (Tip) and *Arabidopsis* (α-Tip), and the vegetative organs of *Arabidopsis* (γ-Tip) [5]. Their homologues (pea 7A and *Arabidopsis* RD28), inducible by water stress, are also

The nucleotide sequence data reported will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession numbers D17443 and D25534.

associated with tonoplast [4, 18]. The evolutionary relationship of Tip proteins indicates their function specialization [5]. One of the possible roles is to present a water-specific channel [8]. Although recent reports suggest that the Mip family is a large, ancient family, no report so far has shown the existence of a member of the Mip family in monocotyledonous plants.

We have been isolating putative genes by random sequencing of rice cDNA clones prepared from anthers and suspension-cultured cells, and under stress conditions [15, 16]. From a cDNA library constructed from rice anther mRNA at the uninucleate microspore stage, we isolated two cDNA clones which showed homology to soybean nod-26 and *Arabidopsis* γ-Tip, respectively. The one, rMip1 (rice Mip 1, 1342 bp) encoded a deduced polypeptide of 284 amino acids. There were 14 duplications of AG sequences in the 5′-untranslated region, followed by the start codon ATG. The other one, rTip1 (rice Tip 1, 1080 bp) encoded 250 deduced amino acids. Southern blot analysis suggested that rMip1 and rTip1 are each encoded by a single-copy gene (data not shown). Hydrophobicity analyses showed that both rMip1 and rTip1 have a hexastyle transmembrane struc-

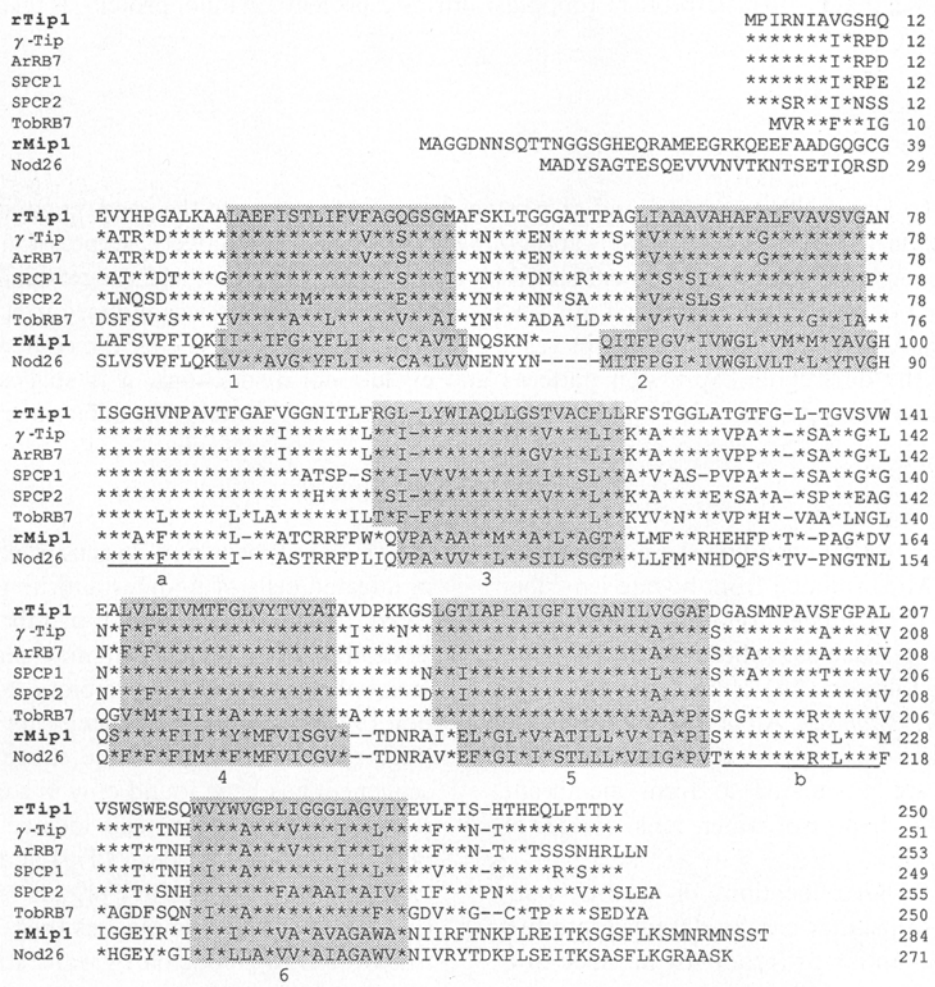

Fig. 1. Comparison of amino acid sequences among rice rTip1, γ-Tip and AtRB7 of *Arabidopsis*, SPCP1 and SPCP2 of soybean, tobacco ToRB7, rice rMip1 and soybean nod-26. Asterisks indicate amino acid identities between rTip1 and other Mip proteins. Two underlined regions (a and b) show the regions highly conserved in Mip family. Six potential hydrophobic domains are shaded and numbered 1–6.

ture, as also present in other Mip proteins (Fig. 1, shaded six regions).

A comparison of the entire amino acid sequences showed that rTip1 shared 79.7%, 78.6%, 75.3%, 74.5% and 64.5% amino acid identity with Tip-type members γ-Tip and AtRB7

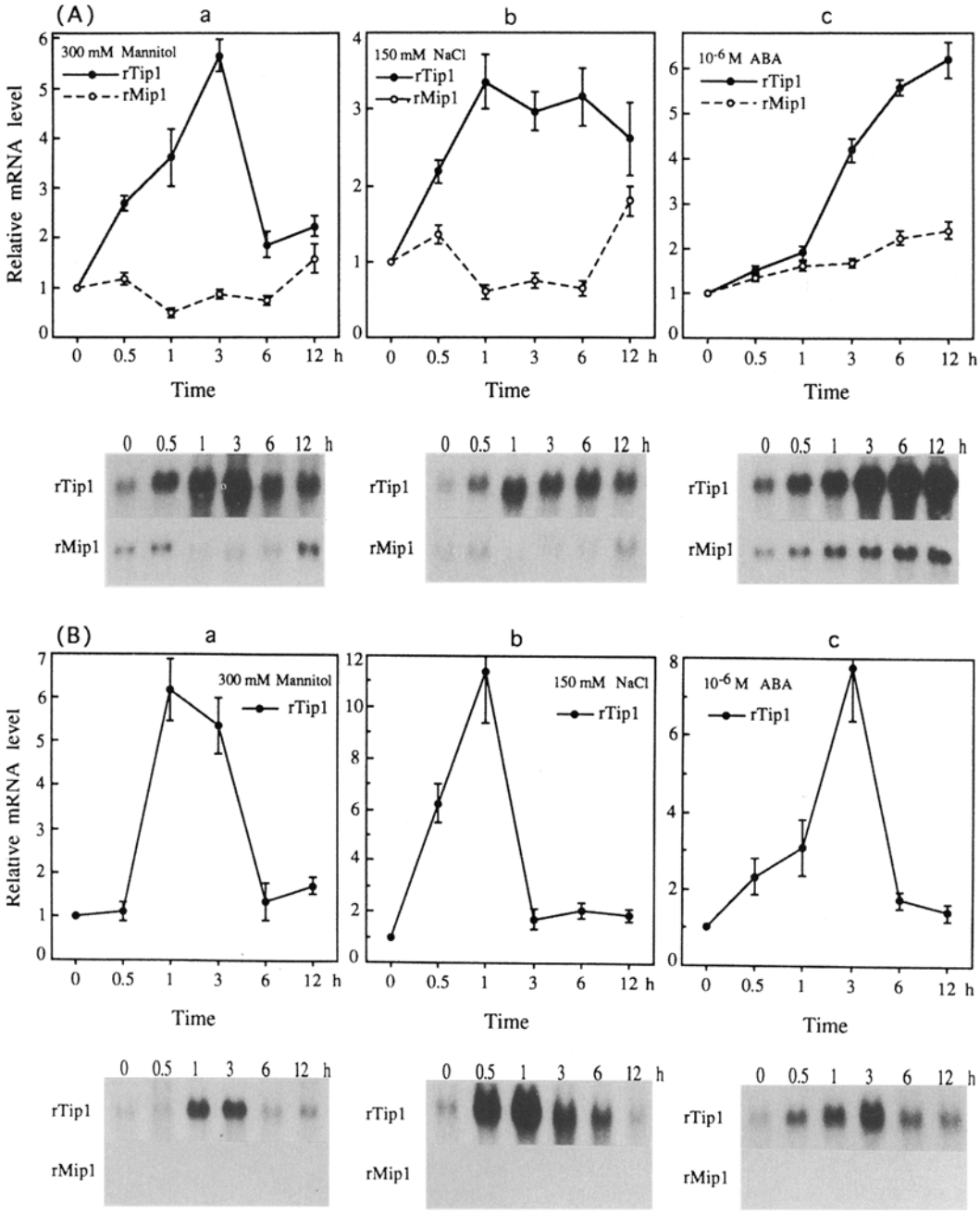

Fig. 2. Relative mRNA accumulation of rTip1 and rMip1 in shoots (A) and roots (B) of rice under water stress, salt stress and exogenous ABA. Ten μg total RNA was electrophoresed and blotted in each lane. A 528 bp *Hin*c II fragment and a 349 bp *Eco* RV fragment from 3′ sides of rTip1 and rMip1 were used as probes for hybridization. The blots were repeated 4–5 times and the standard errors are indicated by vertical bars. A typical autoradiogram is presented below each graph.

[19] of *Arabidopsis*, SPCP1 and SPCP2 of soybean [10], and TobRB7 of tobacco [19], respectively, whereas rMip1 showed 66.2% amino acid homology with nod-26 (Fig. 1). The evolutionary relationship analyzed by UPGMA (unweighted pair group method analysis [20]) using the entire amino acid sequences from plant Mips revealed that rTip1 was closely related to the Tips group, and that rMip1 and nod-26 were closely related in another evolutionary group and distant from Tips (data not shown).

In all of the plant Mip proteins reported so far, there are two highly conserved regions (Fig. 1, underlined a and b). The sequence SGGHXNPAV in the first region is thought to be a 'signature sequence' for plant Mip proteins [5]. This sequence can be used to identify new proteins in the Mip family and to distinguish them from other proteins with six membrane-spanning domains [5]. In rTip1, the corresponding sequence was identical to those in all of the reported plant Mip proteins. However, the corresponding sequence (SGAHXNPAV) in rMip1 differed from those in other plant Mips, but was identical to those in Mip proteins from human [11], animals [3], insects [13] and microorganisms [17]. Thus, rMip1 may represent a novel type of plant Mip.

Northern blot analyses showed similar expression of rTip1 and rMip1 at different developmental stages of anthers (data not shown). However, rTip1 was expressed in both shoots and roots of rice, whereas rMip1 was expressed only in shoots. Figure 2 shows the expression levels of rTip1 and rMip1 in shoots and roots of 7-day old rice seedlings under stress conditions of 300 mM mannitol, 150 mM NaCl and 1 μM M ABA. The expression level of rTip1 increased under water or salt stress. In shoots, the highest level of relative mRNA accumulation (6-fold) was observed after 3 h of water stress (Fig. 2A, a). A 3.5-fold increase in relative mRNA level was detected at 1 h under salt stress (Fig. 2A, b). In roots, the highest levels of relative mRNA accumulation (6.2- and 11.4-fold) appeared at 1 h under water or salt stress (Fig. 2B, a and b).

Changes in expression of the two Tip-type genes (7A and RD28) have been observed under water stress [4, 18]. Based on the evolutional relationship and the expression patterns, rTip, as Tip-type genes, may encode a water-channel, ion-channel or specific-channel protein which is associated with the regulation of cell water when plants are subjected to water stress or salt stress. The inducement of rTip1 may play an important role in protecting desiccated cells by accelerating transport of water, ions or metabolites across cell membrane.

Analysis of mRNA accumulation of rMip1 under stress conditions indicated a different expression pattern. The transcript level of rMip1 in shoots remained relatively stable under water or salt stress, no striking change was found during 12 h time courses (Fig. 2A, a and b). The expression of rMip1 in roots was not induced at all by the same treatments (Fig. 2B, a and b). The different expression patterns and evolutional relationships between rMip1 and rTip1 suggest that the possible channel proteins encoded by these two genes may function in different transport systems.

The Mip genes have different responses to ABA treatment. Desiccation-induced RD28 gene is ABA-insensitive. However, dehydration-induced 7A gene of pea and blue-light induced AthH2 gene of *Arabidopsis* [7] are sensitive to ABA. The increase in gene expression under water or salt stress may be due to the accumulation of ABA in cells. Transcript levels of rTip1 and rMip1 were enhanced by exogenous ABA (Fig. 2A, c and 2B, c), suggesting some role of rTip1 and rMip1 in senescence process.

Although the Mip proteins are believed to play a significant role in osmoregulation [12], their specific functions are not well understood. Thus, further studies such as *in vivo* expression in *Xenopus* oocytes [8] and transgenic approach may facilitate the understanding of specific functions of Mip proteins in plant cells.

Acknowledgements

We gratefully acknowledge the gift of rice anther mRNA from Dr K. Toriyama. Critical reading of

this manuscript by T. W McCaull is appreciated. This research was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture and Science, Japan; a grant from the Rockefeller Foundation and a grant from the Dan Charitable Trust Fund for Research in the Biological Sciences, Japan.

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