The Plant Journal (1998) **13**(5), 685–689

SHORT COMMUNICATION

Isolation and molecular characterization of the *Arabidopsis TPS1* gene, encoding trehalose-6-phosphate synthase

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Summary

An *Arabidopsis thaliana* cDNA clone, *AtTPS1*, that encodes a trehalose-6-phosphate synthase was isolated. The identity of this protein is supported by both structural and functional evidence. On one hand, the predicted sequence of the protein encoded by *AtTPS1* showed a high degree of similarity with trehalose-6-phosphate synthases of different organisms. On the other hand, expression of the *AtTPS1* cDNA in the yeast *tps1* mutant restored its ability to synthesize trehalose and suppressed its growth defect related to the lack of trehalose-6-phosphate. Genomic organization and expression analyses suggest that *AtTPS1* is a single-copy gene and is expressed constitutively at very low levels.

Introduction

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is found widely in the microbial world and in lower invertebrates (Elbein, 1974). However, it is apparently absent in most plants, its presence being restricted to some primitive phyla of vascular plants and to some 'resurrection plants' among the angiosperms (Müller *et al.*, 1995). In micro-organisms, trehalose serves as a protectant against heat and desiccation stress (Van Laere, 1989), probably through the stabilization of protein structure and lipid membranes (Crowe *et al.*, 1987; Hottiger *et al.*, 1994). Attempts have been made to engineer trehalose synthesis in tobacco plants by overexpression of bacterial or yeast trehalose-biosynthetic genes (Goddijn *et al.*, 1997; Holmström *et al.*, 1996; Romero *et al.*, 1997), which have led to an increased drought resistance. However, in some cases, high levels of expression of these heterologous genes are associated with anomalies in carbohydrate metabolism and in the overall development and morphology of the plants (Romero *et al.*, 1997).

In yeast, two genes, *TPS1* and *TPS2,* responsible for trehalose synthesis have been isolated (Bell *et al.*, 1993; De Virgilio *et al.*, 1993; Vuorio *et al.*, 1993). As a first step to characterize the metabolism of trehalose in plants and produce transgenic plants with altered carbohydrate production, we decided to isolate putative *Arabidopsis* genes involved in the synthesis of this disaccharide. We took advantage of the *tps1* mutant from *Saccharomyces cerevisiae*, which is unable to grow in glucose but can grow in galactose (González *et al.*, 1992), to use a complementation strategy as a tool to isolate the *Arabidopsis TPS1* homologue. It has been proposed that the *tps1* mutant phenotype is caused by a loss of regulation of hexokinase by trehalose-6-phosphate (trehalose-6-P) (Blázquez *et al.*, 1993). Here we report the isolation of the *Arabidopsis TPS1* gene (*AtTPS1*) and its molecular characterization. The results presented indicate that, contrary to the commonly accepted idea, *Arabidopsis*, a higher plant, has the ability to synthesize trehalose.

Results and discussion

Isolation of the Arabidopsis TPS1 *gene*

In order to isolate the *Arabidopsis* homologue of *TPS1*, we transformed a *S. cerevisiae tps1* mutant with an *Arabidopsis* cDNA library under the control of the yeast *PGK1* promoter (Minet *et al.*, 1992) and looked for complementation of its growth defect in glucose. Thirty thousand transformants were obtained in galactose and replica plated to glucose. Twenty-seven positive colonies were isolated, all of which carried plasmids with overlapping inserts as assessed by restriction and Southern analysis (data not shown). Plasmid pATT9–1 was selected for further studies as it contained the longer insert. Complementation of the *tps1* mutant growth phenotype (Figure 1a) does not prove by itself that the cloned gene encodes a trehalose-6-P synthase. In fact, extragenic suppressors of *tps1* have been isolated that partially rescue the growth defect only, not other defects associated with the mutation (Blázquez and Gancedo, 1995; Hohmann *et al.*, 1992). Two results argue against pATT9–1 encoding a sup-

Received 28 July 1997; revised 13 November 1997; accepted 13 November 1997.

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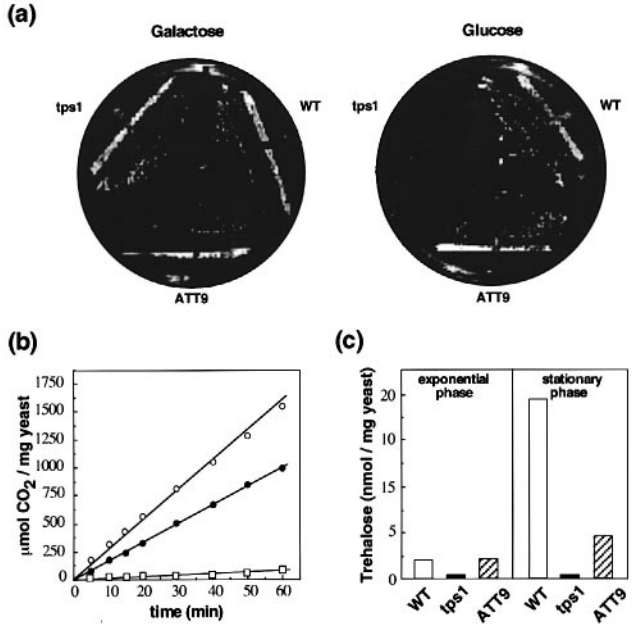

Figure 1. Complementation of the *Saccharomyces cerevisiae tps1* mutant by *Arabidopsis* pATT9–1.
(a) Growth in glucose. Strains W303–1A (WT), WDC-3A (tps1) and WDC-3A transformed with pATT9–1 (ATT9) were grown in rich medium containing 2% galactose or glucose as indicated. WDC-3A cannot grow in glucose, but this defect is complemented by plasmid pATT9–1.
(b) Fermentation of glucose. The production of CO_2 by strains W303–1A (●), WDC-3A (□) and WDC-3A transformed with pATT9–1 (○) was measured as described in the Experimental procedures. Transformation with plasmid pATT9–1 restored the inability of the *S. cerevisiae tps1* mutant to ferment glucose, reaching the same or higher fermentation rate as the wild-type strain.
(c) Trehalose accumulation. Strains W303–1A (WT), WDC-3A (tps1) and WDC-3A transformed with pATT9–1 (ATT9) were grown in minimal medium containing 2% galactose, and samples were harvested during the exponential [4 mg yeast (wet weight) ml^{-1} culture] or the stationary phase [20 mg yeast (wet weight) ml^{-1} culture]. While the wild-type strain accumulated trehalose preferentially in the stationary phase, the *tps1* mutant was unable to synthesize trehalose in either phase of growth. Transformation of the *tps1* mutant with plasmid pATT9–1 allowed accumulation of trehalose on both phases of growth, as expected for the constitutive expression of the *Arabidopsis* cDNA from the yeast *PGK1* promoter.

pressor of the yeast *tps1* mutation. The *tps1* mutant, which cannot ferment glucose because of a block in glycolysis (Blázquez *et al.*, 1993; González *et al.*, 1992), recovered the ability to ferment glucose after being transformed with pATT9–1 (Figure 1b). Furthermore, expression of pATT9–1 restored the capacity to synthesize trehalose in the *tps1* deletion mutant (Figure 1c). These results indicate that plasmid pATT9–1 encodes a protein involved in trehalose synthesis. In wild-type yeast, trehalose synthesis is enhanced during the stationary phase as a result of the activation of the transcription of *TPS1* and *TPS2* (Vuorio *et al.*, 1993). However, in the transformants, trehalose synthesis did not reach the values of the wild-type strain during the stationary phase. This was predictable, since the *Arabidopsis* gene is under the control of a promoter that is not activated by entrance into the stationary phase.

Sequence of the deduced Arabidopsis *TPS1 protein*

Sequence analyses revealed that the insert in plasmid pATT9–1 was a 2.9 kb cDNA that could correspond to the *AtTPS1* full-length cDNA (see below). The longest open reading frame encoded a protein of 942 amino acids, which was 45% longer than that of trehalose-6-P synthases from other organisms. In the first 500 amino acids, the sequence similarity with other TPS proteins lay between 35% and 70% (Figure 2a). The C-terminal portion of the protein has no similarity with other known sequences. All trehalose-6-P synthases and phosphatases show certain degrees of similarity to each other along their sequences. Nevertheless, the protein sequence of *AtTPS1* was clearly more similar to any trehalose-6-P synthase than to the trehalose-6-P phosphatases, as represented in the dendrogram in Figure 2(b). Several *Arabidopsis*-expressed sequence tag (EST) clones present in the databases are identical to *AtTPS1*. However, some other EST clones are only similar to *AtTPS1*, and they might correspond to trehalose-6-P phosphatases or, alternatively, to a second *TPS1* gene. To test this latter possibility, we performed a Southern analysis with genomic DNA from *Arabidopsis* digested with *Bgl*II, *Eco*RI and *Hin*dIII, and with the full-length *AtTPS1* cDNA as a probe (Figure 3a). The results obtained, when compared to the restriction map of the *AtTPS1* cDNA, suggest that there is a single copy of *AtTPS1*. The extra bands in the *Eco*RI and *Hin*dIII lanes, which were not predicted from the sequence of the cDNA, were also visible after high-stringency washes, and were due to the presence of *Eco*RI and *Hin*dIII restriction sites in intron sequences of the *AtTPS1* gene, as detected by PCR (data not shown).

Expression of the Arabidopsis TPS1 *gene*

The expression of the *AtTPS1* gene was analysed by RNA blot hybridization in different organs of *Arabidopsis*, such as roots, leaves, stems and flowers. When using total RNA preparations and the *AtTPS1* cDNA as a probe, no hybridization bands could be detected in any case (data not shown). However, when poly(A)+ RNA preparations (2 mg) were employed in the experiments, a 3-kb transcript hybridizing with the *AtTPS1* cDNA probe was detected at similar levels in all organs analysed (Figure 4). If the rate of degradation of the RNA is similar in all tissues, these results suggest that the expression of *AtTPS1* is low and not organ specific.

The isolation of a gene encoding a trehalose-synthesizing activity in *Arabidopsis* contrasts to previous reports that failed to detect trehalose in most plants (Müller *et al.*, 1995). This apparent discrepancy may be explained by the low expression of the *AtTPS1* gene, the low activity of the encoded TPS protein, or the presence of trehalose-hydrolysing activities in the plant. Evidence supporting the latter idea has been found by Goddijn *et al.* (1997), who have reported

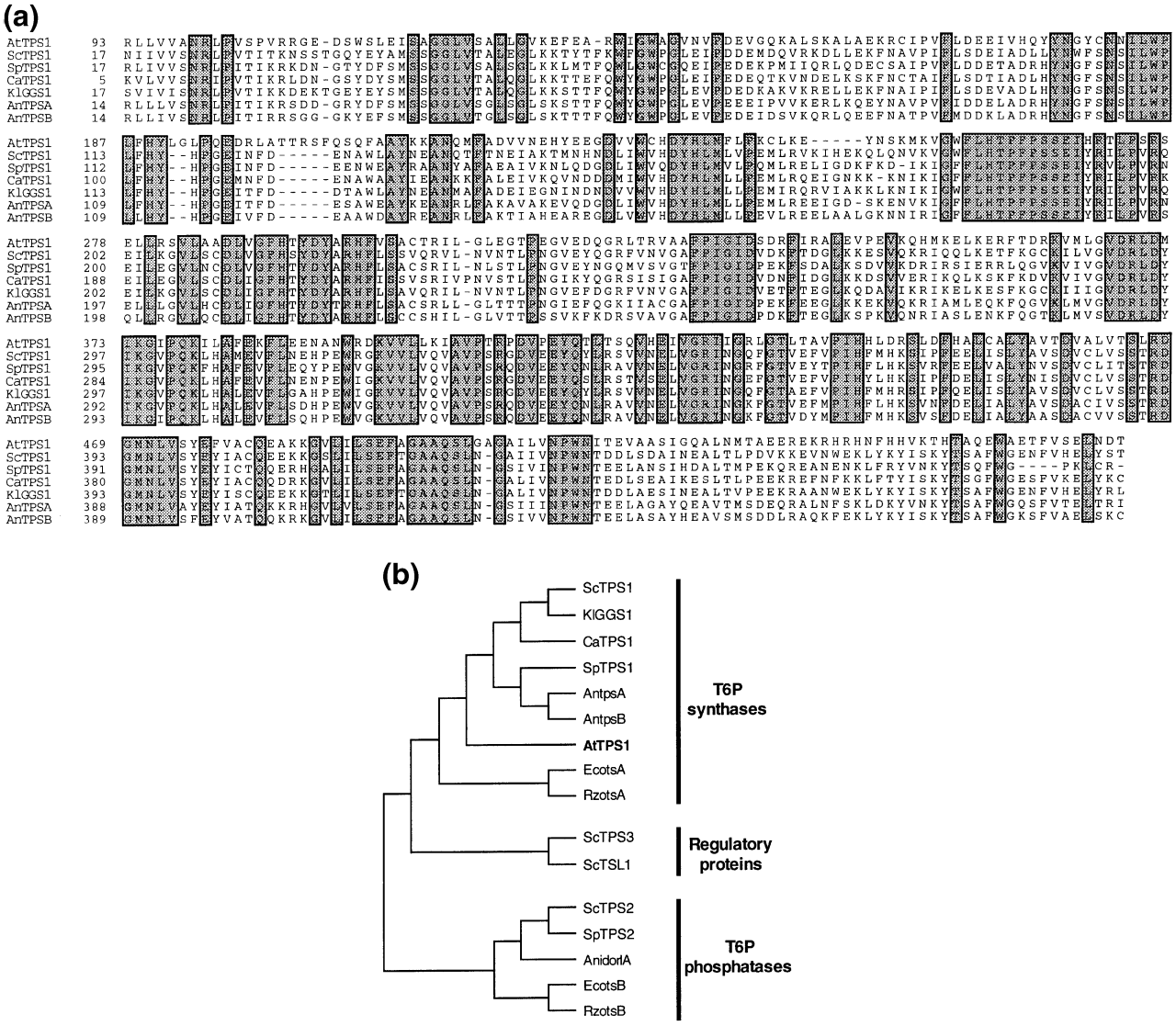

Figure 2. Sequence relationships between trehalose-6-P synthases from different organisms.

(a) Amino acid sequence comparison between an internal region of the *AtTPS1* amino acid sequence and several fungal trehalose-6-P synthases. Identical residues are shaded.

(b) Dendrogram showing the order of pair-wise alignments of trehalose-6-P (T6P) synthase-related amino acid sequences. The dendrogram was generated with the PILEUP program (see the Experimental procedures) using full-length protein sequences, except for SpTPS2, of which only a partial sequence was available.

Sequences are: AtPTS1 (*A. thaliana*, EMBL Y08568), ScTPS1 (*S. cerevisiae*, SwissProt Q00764), SpTPS1 (*Schizosaccharomyces pombe*, SwissProt P40387), KlGGS1 (*Kluyveromyces lactis*, SwissProt Q07158), CaTPS1 (*Candida albicans*, EMBL Y07918), AnTpsA (*Aspergillus niger*, EMBL U07184), AnTpsB (*A. niger*, EMBL U63416), EcotsA (*Escherichia coli*, SwissProt P31677), RzotsA (*Rhizobium* sp., SwissProt P55612), ScTPS3 (*S. cerevisiae*, SwissProt P38426), ScTSL1 (*S. cerevisiae*, SwissProt P38427), ScTPS2 (*S. cerevisiae*, SwissProt P31688), SpTPS2 (*S. pombe*, EMBL D89225), AnidorlA (*A. nidulans*, EMBL X70694), EcotsB (*E. coli*, SwissProt P31678), RzotsB (*Rhizobium* sp., SwissProt P55611).

increased levels of trehalose in tobacco leaves of wild-type plants treated with validamycin A, an inhibitor of trehalases.

The results presented in this paper constitute the first report describing the isolation and characterization of a gene encoding a protein involved in trehalose synthesis in *Arabidopsis*. The presence in the databases of several rice EST clones that are similar to *AtTPS1* suggests that the ability to synthesize trehalose may be extended to other higher plants. Until now, exploitation of trehalose as a stress protectant in plants has been carried out by overexpressing the *TPS1* gene from *Escherichia coli* and yeast (Goddijn *et al.*, 1997; Holmström *et al.*, 1996; Romero *et al.*, 1997). In all cases, plants expressing heterologous trehalose-6-P synthases tolerated desiccation better than the control plants, although severe morphological alterations were observed in those plants having the highest tolerance phenotypes. The availability of the *AtTPS1* gene opens the possibility of understanding the role of trehalose in higher plants and the

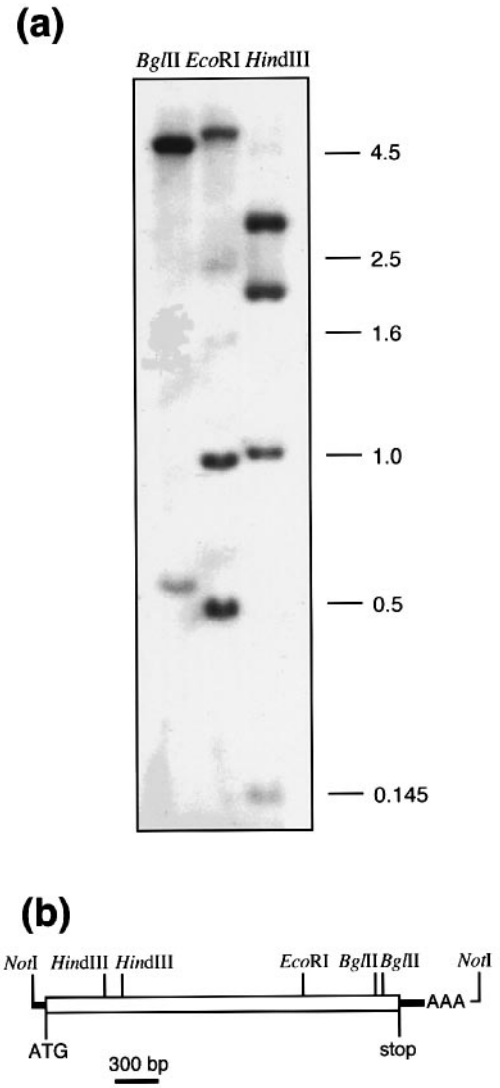

Figure 3. Genome organization of *AtTPS1*.
(a) Southern analysis of the *AtTPS1* genomic region. Genomic DNA was extracted from wild-type Columbia plants and 4 μg were digested with the indicated restriction enzymes. The probe used was the *AtTPS1* cDNA. Hybridization and washes were performed under low-stringency conditions. The sizes of the marker, in kb, are indicated on the right.
(b) Restriction map of the insert in pATT9–1 containing the *AtTPS1* open reading frame.

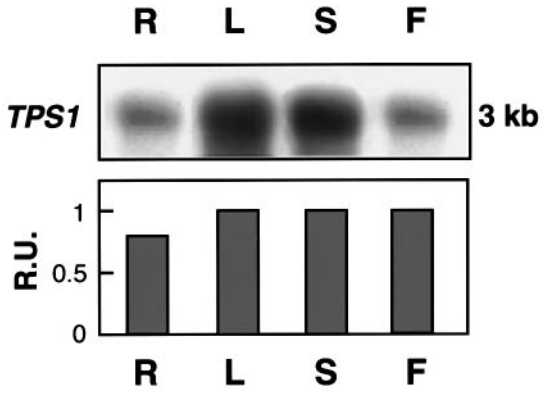

Figure 4. Expression of *AtTPS1* in different organs of *Arabidopsis*. RNA blot hybridizations of 2 mg of poly(A)+ RNA extracted from roots, leaves, stems and flowers of 6-week-old plants. The probe used was a 700-bp DNA fragment corresponding to the 5′ region of the *AtTPS1* cDNA. Histograms represent the quantification of the hybridization signals obtained after hybridization with the *AtTPS1* probe and correction for the loading differences detected with the *RBP4* probe (see the Experimental procedures). The size of the *AtTPS1* transcript is indicated on the right. RU, relative units.

relationships between its synthesis and the carbon metabolism. This information will help to design new strategies to use trehalose to engineer stress tolerance in important crop plants. Experiments to determine these possibilities are underway in our laboratories.

Experimental procedures

Yeast strains and growth conditions

Saccharomyces cerevisiae wild-type strain W303–1A (Thomas and Rothstein, 1989) and the isogenic *tps1* mutant strain WDC-3A (Blázquez *et al.*, 1993) were used throughout the study. Cells were grown in rich or minimal medium with the appropriate auxotrophic requirements, with 2% galactose or glucose, as described previously (Blázquez and Gancedo, 1995).

Plant material

Arabidopsis thaliana plants (ecotype Columbia) were grown at 18°C under long-day conditions (16 h of cool-white fluorescent light, photon flux of 70 mmol m^{-2} s^{-1}), in pots containing a mixture of perlite, vermiculite and sphagnum (1:1:1), and irrigated with water and mineral nutrient solution (Haughn and Somerville, 1986) once a week. Leaf, shoot and flower tissue for Northern analysis was sampled from plants growing on soil for 6 weeks. Root material was obtained from liquid cultures in MS medium (Murashige and Skoog, 1962) containing 2% sucrose.

cDNA library and yeast transformations

A cDNA yeast expression library from *Arabidopsis* under the control of the yeast *PGK1* promoter in the multicopy plasmid pFL61 (Minet *et al.*, 1992) was kindly provided by F. Lacroute (Gif-sur-Yvette, France). Yeast cells were transformed by the lithium acetate procedure (Ito *et al.*, 1983).

DNA and RNA manipulations

DNA manipulations were carried out as described by Sambrook *et al.* (1989). The nucleotide sequence was determined using the dideoxy chain-termination method (Sanger *et al.*, 1977). Sequence data were analysed using the programs of the Wisconsin GCG package (Devereux *et al.*, 1984). Genomic DNA from plants was isolated as described by (Rogers and Bendich, 1988). Total RNA was extracted with the Green Fast-RNA kit (Bio101) according to the manufacturer's indications. Poly(A)+ RNA was isolated with the Pharmacia Micro-mRNA purification kit. The probe used in DNA blot hybridizations was the *AtTPS1* cDNA. The probe used in RNA blot hybridizations was a 700-bp DNA fragment corresponding to the 5′ region of the *AtTPS1* cDNA. As a loading control, we used a

700-bp DNA fragment corresponding to the *Arabidopsis RBP4* gene (Kim *et al.*, 1990). Probes were labelled by random priming with [^{32}P]-dCTP (Feinberg and Vogelstein, 1983). After hybridization, the intensity of the signals was quantified by using a Howteck Scanmaster3+ scanner and Bioimage 3.3 software from Millipore. DNA and RNA samples from each experiment were analysed in at least two independent blots.

Biochemical assays

Trehalose from yeast was extracted as in Kienle *et al.* (1993), and assayed as in Blázquez *et al.* (1994). Fermentation rate was measured with a conventional Warburg respirometer, as described by Blázquez and Gancedo (1995).

Acknowledgements

We thank F. Lacroute (Gif-sur-Yvette, France) for the gift of the *Arabidopsis* cDNA library. This work was supported by fellowships of the Comunidad Autonoma de Madrid (MAB), INIA (ES) and by grants PB94–0091-CO2–01 from the Spanish DGICYT (CG), and SC94–124 from INIA and BIOCT93–0400 from the E.U. (JMZ, JS).

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EMBL Data Library accession number Y08568.