Trehalose metabolism and glucose sensing in plants

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Abstract

Plants sense and respond to changes in carbon and nitrogen metabolites during development and growth according to the internal needs of their metabolism. Sugar-sensing allows plants to switch off photosynthesis when carbohydrates are abundant. These processes involve regulation of gene and protein activity to allow plants the efficient use of energy storage. Besides being a key element in carbon metabolism, glucose (Glc) has unravelled as a primary messenger in signal transduction. It has been proved that hexokinase (HXK) is a Glc sensor. An unusual disaccharide named trehalose is present in very low levels in most plants except for the desiccation-tolerant plants known as ‘resurrection’ plants where trehalose functions as an osmoregulator. We have shown that overexpression of the Arabidopsis trehalose-6-phosphate synthase gene (ATPS1) in Arabidopsis promotes trehalose and trehalose-6-phosphate (T6P) accumulation. Seedlings expressing ATPS1 displayed a Glc-insensitive phenotype. Transgenic lines germinated normally on Glc, in contrast to wild-type seedlings showing growth retardation and absence of chlorophyll and root elongation. Gene-expression analysis in transgenic plants showed up-regulation of several genes involved in sugar signalling and metabolism. These data suggest that ATPS1 and accordingly T6P and trehalose play an important role in the regulation of Glc sensing and signalling genes during plant development.

Introduction

Trehalose (α-D-glucopyranosyl-1, 1-α-D-glucopyranoside) is a non-reducing disaccharide present in bacteria, yeast cells, fungal spores, certain invertebrate species and ‘resurrection’ plants [1]. This sugar accumulates to adapt cells against dehydration, salinity, freezing and heat stress, and it may also serve as a source of energy and carbon [2]. In higher plants and animals the presence of trehalose is rare, although the presence of trehalose biosynthetic genes has been documented in most sequenced genomes by database analysis. There are at least three different pathways for trehalose biosynthesis [3]. The most characterized and common pathway among different organisms involves the formation of T6P (trehalose-6-phosphate) from UDP-Glc and glucose-6-phosphate by trehalose-6-phosphate synthase (TPS), and in a second step the synthesis of trehalose by trehalose-6-phosphate phosphatase (TPP) [4]. In yeast, TPS and TPP form a multimeric enzymatic complex where two other polypeptide chains named Tsl1 (TPS complex large subunit) and Tps3 play a regulatory role [5]. The yeast tps1Δ mutant is unable to grow in Glc as the sole carbon source. There is strong evidence to suggest that this defect is attributable to the additional role of the TPS1 subunit in regulating the flow of Glc into the cell [6]. Also, a correlation has been shown between the inhibition of HxkII by T6P and the control of sugar influx in glycolysis [7].

There is extensive research and patented technology on the use of trehalose as a natural preservative of biological membranes, macromolecules, foods, blood, vaccines and several enzymes, that can be stored and dried at room temperature without loss of structure and bioactivity after rehydration [8]. This observation, and the fact that trehalose accumulates in several organisms to resist extreme stress conditions, led several research groups and companies to select trehalose as a target molecule for genetic engineering of plants. In recent years the overexpression of yeast or bacterial TPS1 genes in plants has been reported to improve stress tolerance in crops. For instance, tobacco, potato and rice plants have an increased drought tolerance after engineering trehalose biosynthesis [9–12]. Nevertheless, transgenic plants expressing a TPS1 gene from Escherichia coli or Saccharomyces cerevisiae with a constitutive promoter display severe morphological alterations such as lancet-shaped leaves, stunted growth, aberrant root development and fewer seeds or sterility. This is likely due to an increased concentration of T6P in the cytosol. To overcome this problem, two molecular genetic improvements have been devised. Firstly, the construction of a translational gene fusion of TPS and TPP coding regions to avoid the unnecessary accumulation of T6P in the cell; and secondly, the use of a stress-inducible promoter

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Abbreviations used: ABA, abscisic acid; ABI4, ABA-insensitive4 gene; AFB3, advene 
phosphoribosyltransferase1 gene; AFG5, Arabidopsis flavonoid pathway ATPS1 gene; AGB1, chlorophyll A/B binding-protein1 gene; gnt2, glucose-insensitive2 mutant; Glc, glucose; HXK, hexokinase; RT, reverse transcriptase; SRTPS1, Selaginella lyrata phosphatase1 gene; SJS-ATPS1, Arabidopsis transgenic lines overexpressing ATPS1 under the SJS promoter; T6P, trehalose-6-phosphate; TPS, trehalose-6-phosphate synthase; Tps3, trehalose-6-phosphate synthase, Tsl1, TPS complex large subunit

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to prevent trehalose sinking during plant growth and development when stress is absent. Accordingly, rice plants have been obtained that show tolerance to different abiotic stresses and without any morphological change, which could compromise plant growth and yield [12]. However, the low levels of trehalose generated in all these transgenic plants suggest that it is unlikely working as an osmoprotectant or membrane stabilizer. Therefore, it is possible that the observed stress tolerance is the product of pleiotropic effects caused by an alteration of trehalose metabolism, and thus this pathway might play a regulatory role.

There are 11 TPS genes in Arabidopsis thaliana and para- doxically trehalose is almost undetectable in this plant [13,14]. The mutation of AtTPS1 (Arabidopsis thaliana TPS gene) by insertion mutagenesis in Arabidopsis provokes an embryo lethal phenotype and suggests that T6P is indispensable for plant growth, development and flowering [15,16]. In addition, it has been shown that T6P regulates carbohydrate utilization and growth via control of glycolysis in a similar manner to yeast [17,18].

The TPS1 homologue genes from Arabidopsis and the resurrection plant Selaginella lepidophylla are able to complement the tps1 yeast mutant [19,20]. Moreover, expression in yeast of SITPS1 (Selaginella lepidophylla TPS gene) complements yeast although trehalose is almost undetectable, suggesting that the Tps1 protein per se is also important to restore cell growth in Glc [20]. Plant TPS1 genes encode large polypeptides compared to their microbial homologues and have N-terminal and C-terminal amino acid extensions absent in bacterial and yeast TPS sequences, which possibly have a regulatory role. We have shown that the deletion of the N-terminal region of Arabidopsis and Selaginella TPS1 genes substantially increased enzyme activity and trehalose accumulation when expressed in yeast and plants [21]. To gain further understanding of the role of trehalose in stress tolerance and regulation of carbohydrate metabolism, we studied the effect of overexpressing the AtTPS1 gene in Arabidopsis.

Material and methods

Plant material

Arabidopsis thaliana Col-0 ecotype was used for overexpressing the AtTPS1 gene. Plants were routinely grown on synthetic soil at 24/20°C with 16-h light/8-h dark cycle. Surface-sterilized seeds were germinated on Murashige and Skoog basal salt mixture medium supplemented with 1% sucrose, B5 vitamins and 0.8% phytoagar. To break dormancy seeds were incubated at 4°C for 4 days with cool-white illumination (20 μE·m⁻²·s⁻¹). Arabidopsis seeds were germinated in 6% Glc and 7-day-old seedlings were collected for RNA extraction.

Plant transformation

The AtTPS1 cDNA was obtained from Arabidopsis total RNA reverse transcribed with Superscript II (Invitrogen) using oligo(dT) primer and amplified by PCR using Expand™ High Fidelity PCR System (Roche) with specific primers derived from sequence in databases. The cDNA insert was cloned in pBluescript SK− (Stratagene) and its DNA sequence was checked, before subcloning in pBlm19 vector, containing the 0.8 kb 35S promoter and 0.3 kb nopaline synthase polyadenylation site. The construct was introduced by electroporation in Agrobacterium tumefaciens C58C1 strain containing the pGV2260 plasmid. The resulting bacteria were used to transform Arabidopsis by in planta vacuum infiltration [22]. Transgenic seedlings were selected on MS media containing 50 μg·ml⁻¹ kanamycin (Sigma). One-week-old seedlings were transferred to pots under the indicated conditions until plants formed seeds. Homozygous lines from the T₁ generation were used in the present work.

Reverse transcriptase-PCR

Reverse transcriptase (RT)-PCR experiments were performed using 5 μg of total RNA extracted as described before from Arabidopsis tissues and used for first strand cDNA synthesis with SuperScript II reverse transcriptase (Invitrogen) and oligo(dT). PCR was conducted at the linear phase of the exponential reaction for each gene after comparison of the PCR products at different cycles. Gene-specific primers derived from database entries to amplify the corresponding gene fragment from Col-0 ecotype for both wild-type or transgenic Arabidopsis were used. RT-PCR products were resolved in 1× TAE, 1% agarose gels stained with ethidium bromide. The bands shown represent the negative of the fluorescent images and a densitometric quantification using Quantity One software (BioRad) of the RT-PCR reaction was performed and normalized for each gene band using the APT1 gene as a control.

Results and discussion

The overexpression of AtTPS1 gene in Arabidopsis was conducted to test for possible effects on stress, development and gene regulation. Therefore, Arabidopsis was transformed with AtTPS1 cDNA and several independent 35S:AtTPS1 (Arabidopsis transgenic lines overexpressing AtTPS1 under the 35S promoter) plants were obtained after transformation with the Agrobacterium tumefaciens system. Homozygous plants were selected from each transgenic line containing a single gene insertion after genetic analysis using kanamycin to score a 3:1 segregation ratio. The expression of AtTPS1 gene was checked by RT-PCR. The trehalose and T6P concentration was determined in 35S:AtTPS1 lines and wild-type seedlings, showing a low increase in both disaccharides in the different transgenic lines. A detailed analysis of possible changes in organ shape or size and plant growth habit was monitored in all the different transgenic lines during their whole life cycle. No morphological changes in individual organs or at the whole plant level were observed in any of the transgenic lines, except for delayed flowering time (1 to 2 weeks) in all of them. Nevertheless, the germination rate was higher in plants overexpressing AtTPS1 compared to wild-type seedlings and similar to abi4 (abscisic acid-insensitive)
mutant. Further characterization of transgenics involved stress tolerance tests in each of the 35S::AtTPS1 lines and wild-type plants. Four-week-old plants were left for 2 weeks without watering. Most transgenic lines recovered from water deprivation after rewatering for 1 day, and recovered full shape whereas wild-type plants did not survive this treatment. These results are similar to other reports where overexpression of TPS1 genes in plants of micro-organisms has been carried out to improve their drought tolerance and suggest that the low concentration of trehalose detected in transgenic plants is probably working more as a signal for stress tolerance rather than as an osmoprotectant [9–11].

Recently, it has been shown that T6P is a signal molecule involved in the control of carbon metabolism in connection with growth and development [17]. Therefore, we tested whether the overexpression of AtTPS1 in Arabidopsis would lead to a sugar response phenotype. Our results showed that Arabidopsis seedlings overexpressing AtTPS1 display a Glc-insensitive phenotype allowing them to normally germinate and develop in a media containing 6% Glc. A similar phenotype was observed in Arabidopsis plants with antisense expression of HXK1 gene and in the Arabidopsis gin2 (Glc-insensitive) mutant of the same gene [23,24]. In recent years, it has been established that sugars, such as Glc, signal to plant development and growth during germination by modulating the overall carbon status in a complex and still unexplained cross-talk with plant hormones such as ethylene and ABA (abscisic acid) [25]. There is clear evidence that HXK1 is a Glc sensor [23,24]. Thus, our results suggest that the AtTPS1 gene has also a role in sugar signalling during vegetative development. Given the observed Glc-insensitive phenotype, we decided to analyse the expression pattern in 35S::AtTPS1 plants of genes involved in Glc signalling and photosynthesis, namely HXK1 and CAB1 (chlorophyll A/B binding protein1) genes. Firstly, as expected, an increase in the AtTPS1 transcript in plants overexpressing this gene was observed (Figure 1). The APT1 (adenine phosphoribosyltransferase1 gene) was used as a constitutive control because it is expressed at similar levels in plants grown with or without Glc, and no altered gene expression is observed in transgenic lines compared to wild-type plants. An induction of HXK1 and CAB1 genes was observed in transgenic plants grown in media without Glc (Figure 1). When seedlings were germinated in the presence of Glc, CAB1 expression was not repressed in 35S::AtTPS1 plants in comparison with wild-type plants, which showed a significant decrease in CAB1 transcription. In contrast, HXK1 transcript was repressed in plants overexpressing AtTPS1 versus wild-type grown in the presence of Glc. We have also showed that overexpression of AtTPS1 provoked an increase in ABI4 (ABA-insensitive4 gene) transcription in plants germinated without Glc. In a similar manner to the effect on HXK1, ABI4 expression in 35S::AtTPS1 plants drops significantly when Glc was present (results not shown). These results suggest that trehalose is a synergistic modulator of sugar-mediated gene expression and it is consistent with the observed Glc-insensitive phenotype of 35S::AtTPS1 plants.

It has been reported that Arabidopsis HXK1 antisense plants have a delayed senescence and increased stress resistance [26]. We have observed an absence of AtTPS1 expression in HXK1-antisense plants, thus suggesting the dependence of AtTPS1 expression on the presence of HXK1. These results suggest that AtTPS1 might participate in HXK-dependent pathway in a complex regulatory network.

A higher expression of CAB1 in 35S::AtTPS1 seedlings is consistent with previous observations that plants overexpressing TPS1 have dark-green leaves and higher photosynthetic rates [27]. However, it has been reported that overexpression of the E. coli TPS1 gene leads to a Glc-sensitive phenotype [17]. As already mentioned an important difference between microbial and plant TPS1 gene products is the presence of an N-terminal extension of about 80 amino acids in length. Thus, it is probable that besides T6P, the
Proposed model of regulatory interactions among Glc, stress, HXK1, TPS1 and ABI4

Solid arrows represent cross-regulation and dotted lines cross-regulation in the presence of Glc.

N-terminal region of AtTPS1 is also part of the signalling process. In addition, since trehalose did not accumulate at significant levels, the drought tolerance phenotype in plants overexpressing AtTPS1 could be a consequence of an altered regulation of the Glc signalling process.

A model of the possible mechanism of AtTPS1 in Glc signalling is shown in Figure 2. Glc is a central key to several signalling and regulatory pathways and integrates external cues to adapt cells to abiotic stress, growth and development. AtTPS1 is part of the HXK1 signalling pathway and in turn controls ABI4, although in the presence of Glc AtTPS1 is a negative modulator of both HXK1 and ABI4. ABI4 and AtTPS1 have opposite effects on germination, shoot development, cotyledon expansion and greening. There is evidence supporting the notion that abiotic stress induces AtTPS1 expression [28], whereas it is possible that HXK1 gene might be repressed by stress as can be inferred from current data [26]. AtTPs1 and/or T6P and/or trehalose might influence gene regulation at different molecular levels including transcription, mRNA stability, translation and/or protein modifications. Another molecular actors in this network might be the 14-3-3 proteins, which are known to interact with phosphoserine in diverse proteins including TPS [29]. After signalling, TPP would convert T6P into trehalose, which would rapidly be degraded to Glc by trehalase. The turnover of T6P to yield Glc via trehalose would avoid unnecessary sinking of this disaccharide. Therefore, an important role of the trehalose biosynthesis pathway in higher plants would be the synthesis of small amounts of the signalling molecules T6P and trehalose rather than accumulation of trehalose as an osmoprotective compound. This could explain why trehalose generally does not accumulate in significant quantities in higher plants, although the capacity for its biosynthesis has been retained during evolution.

References


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