The Role of Trehalose Biosynthesis in Plants

The role of trehalose in plants is a curious one. Originally believed to serve as an osmoprotectant, its presence in plants at very low levels makes this role unlikely. This month's *High Impact* article by Avonce et al., titled "The Arabidopsis Trehalose-6-P Synthase *AtTPS1* Gene Is a Regulator of Glucose, Abscisic Acid, and Stress Signaling," appeared in our November 2004 issue and adds to the growing body of evidence that it is not trehalose itself but more likely a synthesis pathway intermediate or perhaps an enzyme of the synthesis pathway that plays a key role in plants.

BACKGROUND

Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is a nonreducing disaccharide formed through a 1-1 alpha bond linking two Glc moieties. A variety of organisms synthesize this compound, including plants, fungi, bacteria, and invertebrate animals. Trehalose is the main blood sugar in insects and serves as a major energy storage molecule enabling flight. In those plants that accumulate trehalose, it is most commonly believed to aid in their ability to survive extended periods of desiccation. However, with few exceptions, this is unlikely to stem from a direct role of trehalose itself since only trace amounts of trehalose are present in most (angiosperm) plants. Current studies are defining a role for the trehalose precursor, trehalose-6-P (T6P), as a regulatory molecule, especially in sugar influx and metabolism (for review, see Eastmond and Graham, 2003). Arabidopsis (*Arabidopsis thaliana*) plants with an insertion in *AtTPS1* do not develop mature embryos, stressing the importance of the trehalose synthesis pathway in embryo maturation and development (for review, see Eastmond and Graham, 2003). Recently, T6P has been implicated in the redox activation of ADP-Glc phosphorylase, the enzyme that catalyzes the first committed step of starch synthesis (Kolbe et al., 2005). Also, T6P has been recognized as a key molecule enhancing photosynthetic capacity and thus offering a long-sought dream of agronomists: improvement of crop biomass (Pellny et al., 2004).

Multiple trehalose biosynthetic pathways have been identified in bacteria and archeae, but only one has been found in the eukaryotes examined to date (Avonce et al., 2006). The eukaryotic pathway shares many similarities to Suc synthesis in plants (for review, see Goddijn and van Dunn, 1999). In the first step, UDP-Glc and Glc-6-P are linked by T6P synthase (TPS) to form T6P. The phosphate group is removed by T6P phosphatase (TPP), resulting in trehalose. Trehalose is subsequently broken down into two molecules of Glc by trehalase. In Arabidopsis, 11 copies of TPS and 10 of TPP are present, and all appear to be expressed in a tissue-specific and developmentally controlled manner (Avonce et al., 2006). The *AtTPS* genes are also differentially expressed under conditions of elevated Glc (Price et al., 2004), further underscoring the potential importance of the intermediate synthesis product T6P.

WHAT WAS SHOWN

In the study by Avonce et al. (2004), *AtTPS1*, one of 11 *TPS* genes in Arabidopsis, was overexpressed in Arabidopsis under the control of the *35S* promoter. Despite the accumulation of higher levels of *AtTPS1* mRNA and protein than wild type, trehalose did not appreciably accumulate in these plants. This was similar to what was seen in earlier studies in tobacco (*Nicotiana tabacum*; Goddijn et al., 1997) and Arabidopsis (Schluepmann et al., 2003) expressing bacterial *TPS1*. T6P, the synthesis intermediate and product of TPS, was found at higher levels than wild type.

Increased drought tolerance (relative to wild type) was also observed in the *AtTPS1*-overexpressing plants. The transgenic lines had higher relative water content than wild type and, unlike wild type, were able to recover from 2 weeks of water deprivation after a return to the normal watering regime. The authors noted that, since trehalose did not accumulate to high levels in these plants, the increase in drought tolerance was not likely due to a direct effect of trehalose but more likely due to other changes associated with *AtTPS1* overexpression.

The overexpression of *AtTPS1* caused no morphological changes other than delayed flowering, in contrast to previous studies where plants overexpressing bacterial or yeast *TPS1* had altered morphology. One major difference between *AtTPS1* and bacterial or yeast *TPS1* is the presence of the N-terminal and/or C-terminal regions that could possibly account for the difference between these studies. Previous work from this lab had demonstrated that deletion of the N-terminal regions of AtTPS1 resulted in higher catalytic activity of the enzyme (Van Dijck et al., 2002).

A Glc-insensitive phenotype was observed in *AtTPS1*-overexpressing plants, allowing seeds to germinate on Murashige and Skoog (MS) plates supplemented with 6% Glc, whereas wild-type seeds had poor germination rates under the same conditions. This group used this observation as a basis to develop a process to use AtTPS1 as a selectable marker for obtaining transgenic plants and thus avoid the use of antibiotics (Leyman et al., 2006).When seeds were germinated on varying concentrations of abscisic acid

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(ABA), more of the seeds overexpressing *AtTPS1* germinated than wild type, suggesting insensitivity also to ABA. To determine if the Glc-insensitive phenotype was due to altered ABA levels in the transgenic plants, ABA levels in seedlings were determined. No difference was observed between wild type and the overexpressing plants when germinated on MS medium. However, when the MS medium was supplemented with Glc, ABA levels increased in wild-type plants, consistent with previous reports (Arenas-Huertero et al., 2000), while remaining constant in the *AtTPS1*-overexpressing plants, suggesting an interaction between AtTPS1 gene expression and ABA metabolism. To further explore the interactions between AtTPS1 overexpression and ABA and Glc, the expression pattern of genes known to be regulated by ABA or Glc was analyzed in the *AtTPS1*-overexpressing plants. Seedlings germinated on MS medium containing Glc had decreased gene expression of *ABI1* (ABA signal transduction), *HXK1* (sugar signaling component), and *ApL3* (starch biosynthesis).

The product of TPS, T6P, is a known signaling molecule in yeast (*Saccharomyces cerevisiae*) and is believed to inhibit hexokinase, thus regulating the entry of Glc and Fru into glycolysis (Blázquez et al., 1998). In this study by Avonce et al. (2004), *AtTPS1* overexpression led to Glc- and ABA-insensitive phenotypes as well as alterations in the expression of genes known to be regulated by sugars, suggesting a link between *AtTPS1* overexpression and sugar sensing.

THE IMPACT

Detection of trehalose and T6P in plants is a potential problem given that both are present at such low levels, potentially leading to inaccurate measurements. Lunn et al. (2006) developed a very sensitive assay to measure T6P in the femto-picomole range. They then used this assay to measure T6P levels in Suc-starved Arabidopsis plants after Suc addition and found that T6P levels increased with the sugar concentration. Additionally, they found that increasing levels of sugars and T6P led to a change in the redox status of ADP-Glc pyrophosphorylase and a stimulation of starch synthesis in vivo. This result lent further support to the notion that T6P acts as a regulatory molecule of the sugar status of a plant, defined by the Avonce et al. (2004) study, as well as the role of T6P in mediating Suc-induced changes in the rate of starch synthesis (Kolbe et al., 2005). The work by Avonce et al. (2004) was the first time that trehalose metabolism was linked to downstream changes in gene expression, further supporting its possible role as a second messenger.

Gómez et al. (2006) found another possible role of AtTPS1 while studying seed development in the *tps1* mutant, a lesion typically characterized as embryo lethal. These mutants are arrested at the torpedo stage of embryo development, but Gómez et al. (2006) found that if incubated for an extended period on agar the seeds germinated. These seedlings have limited growth and remain very small plantlets that do not progress beyond the vegetative stage. A more detailed examination of the *tps1* embryos revealed thicker cell walls than wild type, which the authors hypothesized could be due to a change in sugar nucleotide metabolism. Cell division could also be reduced in the mutants, possibly due to thickened cell walls or, alternatively, due to the presence of trehalose as sugar availability has been proposed to be a regulator of G1 phase of cell division in cell cultures. This suggests a role for TPS1 during embryo development in the coordination of cell wall biosynthesis and cell division with metabolism.

CONCLUSION

It would be perplexing that Arabidopsis has 11 copies of TPS and 10 of TPP genes if a product of the biosynthesis pathway were not important to some aspect of the plant's life cycle or survival. Studies by Avonce et al. (2004) and others have demonstrated the importance of TPS to plant growth and survival, and more recent works have shown the importance of T6P. These studies, together with the low levels of trehalose present in the majority of plants, support the very important role of T6P for plant growth, development, and stress tolerance.

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