A Tale of Two Sugars: Trehalose 6-Phosphate and Sucrose[1][OPEN]

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Trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is an essential signal metabolite in plants, linking growth and development to carbon status. The Suc-Tre6P nexus model postulates that Tre6P is both a signal and negative feedback regulator of Suc levels, forming part of a mechanism to maintain Suc levels within an optimal range and functionally comparable to the insulin-glucagon system for regulating blood Glc levels in animals. The target range and sensitivity of the Tre6P-Suc feedback control circuit can be adjusted according to the cell type, developmental stage, and environmental conditions. In source leaves, Tre6P modulates Suc levels by affecting Suc synthesis, whereas in sink organs it regulates Suc consumption. In illuminated leaves, Tre6P influences the partitioning of photoassimilates between Suc, organic acids, and amino acids via posttranslational regulation of phospho*enol*pyruvate carboxylase and nitrate reductase. At night, Tre6P regulates the remobilization of leaf starch reserves to Suc, potentially linking starch turnover in source leaves to carbon demand from developing sink organs. Use of Suc for growth in developing tissues is strongly influenced by the antagonistic activities of two protein kinases: SUC-NON-FERMENTING-1-RELATED KINASE1 (SnRK1) and TARGET OF RAPAMYCIN (TOR). The relationship between Tre6P and SnRK1 in developing tissues is complex and not yet fully resolved, involving both direct and indirect mechanisms, and positive and negative effects. No direct connection between Tre6P and TOR has yet been described. The roles of Tre6P in abiotic stress tolerance and stomatal regulation are also discussed.

TREHALOSE 6-PHOSPHATE EMERGES FROM OBSCURITY

Our tale begins with the emergence of trehalose 6-phosphate (Tre6P) from obscurity to its recognition as an essential signal metabolite in plants, with influence on growth and development that rivals any other signaling molecule, including the major phytohormones (Fig. 1). Trehalose (α-D-glucopyranosyl-1,1-α-D-glucopyranoside) is a nonreducing disaccharide that is found in all major groups of organisms except vertebrates and has various functions: osmolyte, storage reserve, transport sugar, and stress protectant. There are five known pathways of trehalose synthesis in prokaryotes, but only one of these is found in eukaryotes (Avonce et al., 2006). In this two-step pathway, a phosphorylated intermediate, Tre6P, is first synthesized from UDP-Glc (UDPG) and Glc 6-phosphate (Glc6P) by Tre6P synthase (TPS), and then dephosphorylated to trehalose by Tre6P phosphatase (TPP; Cabib and Leloir, 1958). For over a century it has been known that some primitive plants, such as *Selaginella lepidophylla*, are able to produce trehalose (Anselmino and Gilg, 1913), and later studies reported the presence of trehalose in chlorophyte algae, mosses, liverworts, and ferns (Elbein, 1974; Kandler and Hopf, 1980). However, for many years, the existence of trehalose in flowering plants was questioned by most authorities, with a

ADVANCES

- The Suc-Tre6P nexus concept postulates that Tre6P is both a signal and a negative feedback regulator of Suc levels in plants, helping to maintain Suc levels within an optimal range for the plant.
- In source leaves, Tre6P influences photoassimilate partitioning between Suc and organic and amino acids during the day, acting via posttranslational regulation of PEP carboxylase and nitrate reductase. At night, Tre6P regulates transitory starch breakdown in leaves, potentially linking starch turnover to the demand for Suc from growing sink organs.
- In sink organs, Tre6P regulates Suc consumption and growth, probably acting via multiple mechanisms, including inhibition of SnRK1.
- Tre6P and trehalose metabolism in guard cells play a crucial role in control of stomatal function.
- Targeted manipulation of Tre6P can improve abiotic stress tolerance and yield in crop plants.

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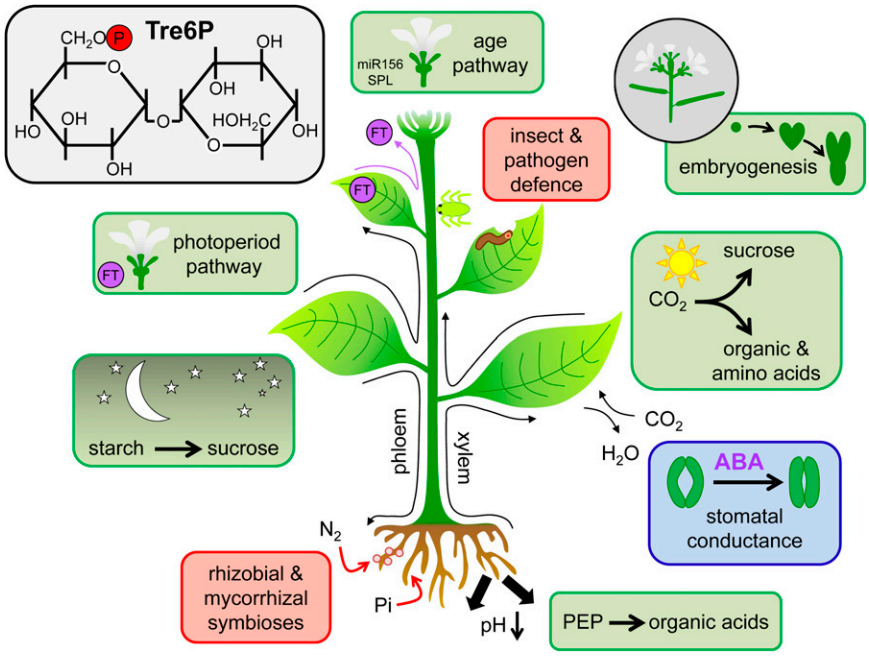

Figure 1. Tre6P signaling in plants. Tre6P is the phosphorylated intermediate of trehalose biosynthesis. It is a signal of Suc status in plants and influences many metabolic and developmental processes, either directly (green boxes) or indirectly as the precursor of trehalose (red boxes). Both Tre6P and trehalose are implicated in regulation of stomatal conductance (blue box). The central image is based on a drawing downloaded from www-plb.ucdavis.edu/labs/lucas/fig4.jpg.

prevailing consensus that any trehalose detected in angiosperms must have originated from microbial contamination or was an analytical artifact (Gussin, 1972). Thus, angiosperm trehalose (and by implication Tre6P) was as mythical as the unicorn, or the legendary snark (Carroll, 1898), and only the brave, or foolhardy, went looking for it.

Undaunted, a few pioneers persisted and were eventually rewarded by finding trehalose in several desiccation-tolerant angiosperms known as "resurrection plants" (e.g. *Myrothamnus flabellifolius* and *Sporobolus* spp.), in such large amounts that it could not be dismissed as microbial contamination (Drennan et al., 1993; Iturriaga et al., 2000). Nevertheless, trehalose synthesis and Tre6P were still generally believed to be absent, or at least of little interest, in other angiosperms, until two parallel developments shattered this consensus. The first was the finding of genes encoding catalytically active TPS (Blázquez et al., 1998) and TPP (Vogel et al., 1998) enzymes in Arabidopsis (*Arabidopsis thaliana*), a desiccation-intolerant plant. The second was the observation of unexpected phenotypes (e.g. altered leaf shape and delayed senescence) in plants engineered to express microbial enzymes for trehalose production (Goddijn et al., 1997; Romero et al., 1997; Pilon-Smits et al., 1998; Goddijn and van Dun, 1999). Together, these discoveries demonstrated that the capacity to synthesize trehalose is not limited to resurrection plants among the angiosperms and revealed for the first time the unexpected and far-reaching effects of tinkering with trehalose metabolism in plants.

TPS and *TPP* genes have now been identified in species from all major plant taxa, indicating that trehalose metabolism is probably universal in the plant kingdom (Avonce et al., 2006, 2010; Lunn, 2007; Lunn et al., 2014). Arabidopsis has 11 *TPS* (*AtTPS1-AtTPS11*) and 10 *TPP* (*AtTPPA-AtTPPJ*) genes (Arabidopsis Genome Initiative, 2000; Leyman et al., 2001). Similarly large *TPS* and *TPP* gene families are present in other flowering plants, and there has been independent duplication within these gene families in different plant lineages (Lunn, 2007; Ramon et al., 2009; Vandesteene et al., 2010, 2012). As will be discussed in more detail below, AtTPS1 is the predominant, but not the only, catalytically active TPS in Arabidopsis (Delorge et al., 2015). Arabidopsis *tps1* null mutants (e.g. *tps1-1* and *tps1-2*) fail to complete embryogenesis, becoming arrested at the torpedo stage (Eastmond et al., 2002; Gómez et al., 2006), but can be rescued by dexamethasone-inducible expression of *AtTPS1* during embryogenesis (van Dijken et al., 2004) or by expression of *AtTPS1* under the control of an embryo-specific (*ABI3*) promoter to give viable seeds (Gómez et al., 2010). However, after germination, *tps1* plants remain stunted and flower late, or not at all. In maize (*Zea mays*), loss of the RAMOSA3 isoform of TPP, which is specifically expressed in parts of the floral primordia, leads to abnormal branching of the male and female inflorescences in the *ramosa3* mutant (Satoh-Nagasawa et al., 2006). These observations showed that the pathway of trehalose synthesis via Tre6P is essential for normal growth and development at all of the major stages in a plant's life cycle: seed development, vegetative growth, and flowering.

Heterologous expression of microbial *TPS* and *TPP* genes in plants played a key role in unlocking the mystery of why interfering with trehalose biosynthesis has such a profound effect on plant growth and development. Early reports from plants engineered to produce trehalose noted that even those with the most

abnormal phenotypes contained barely detectable levels of trehalose (Goddijn et al., 1997; Romero et al., 1997; Pilon-Smits et al., 1998; Goddijn and van Dun, 1999). Then a breakthrough came from comparison of Arabidopsis plants expressing the *Escherichia coli* TPS (otsA) or TPP (otsB) under the control of the constitute CaMV 35S promoter (Schluepmann et al., 2003). The *35S:otsA* plants had small leaves, flowered early, and produced highly branched inflorescences, whereas the *35S:otsB* plants had large leaves, flowered late, and had few branches. The opposite effects of TPS and TPP overexpression suggested that changes in the level of Tre6P, rather than trehalose, were the cause of the phenotypes. This was elegantly confirmed by expression of the *E. coli* phosphotrehalase, which cleaves Tre6P to Glc6P and Glc (thereby decreasing Tre6P levels without increasing trehalose) and gave the same phenotype as overexpression of TPP, whereas overexpression of the *E. coli* cytoplasmic trehalase (to decrease trehalose) had little obvious effect on plant morphology (Schluepmann et al., 2003). Thus, Tre6P emerged from mythical obscurity into the limelight, no longer a metabolic curiosity of doubtful existence but a molecule of great interest.

MEASURING Tre6P: THE TECHNICAL CHALLENGE OF WORKING AT THE FEMTOMOLE SCALE

Once Tre6P was identified as a molecule worth studying, finding a way to measure it reliably in plant tissues became a high priority. Although not quite homeopathic, the amounts of Tre6P in plant tissues turned out to be extremely low (typically between 10 and 1000 pmol g^{-1} fresh weight in Arabidopsis; Lunn et al., 2014) and proved difficult to measure using existing assays. These included a method based on inhibition of a Tre6P-sensitive hexokinase from yeast (*Saccharomyces cerevisiae*), which can be used to quantify Tre6P in yeast cells, but lacked the sensitivity and specificity needed for reliable measurement of Tre6P in plant tissues, where levels are up to 100 times lower. New approaches were needed, and HPLC coupled to tandem mass spectrometry (LC-MS/MS) emerged as the most promising technique, offering both high sensitivity and high specificity.

Baseline separation of Tre6P from its most common isomer, Suc 6′-phosphate (Suc6P; the intermediate of Suc biosynthesis) was first achieved using anion-exchange chromatography for the LC phase (Lunn et al., 2006). Coupling to a triple quadrupole mass spectrometer provided additional specificity and reliable quantification down to about 100 fmol (100×10^{-15} mol; Lunn et al., 2006). With technical advances in mass spectrometry, sensitivity has been increased 50-fold to give a current detection limit of 2 fmol (2×10^{-15} mole; Figueroa et al., 2016). The anion-exchange LC-MS/MS assay was validated by recovery experiments, and its specificity was demonstrated by preincubating plant extracts with purified, recombinant TPP or Suc6P phosphatase, which specifically removed the mass spectrogram peaks assigned to Tre6P and Suc6P, respectively (Lunn et al., 2006). The standards used for calibration of the LC-MS/MS assay had to be independently verified by enzymatic analysis, due to the low purity (60–70%) of the Tre6P stocks that were commercially available at the time (for further discussion, see Yadav et al., 2014; Lunn et al., 2014), and all samples were spiked with a deuterium-labeled internal Tre6P standard to correct for ion suppression and other matrix effects (Lunn et al., 2006).

LC-MS/MS and LC-MS-based methods have now become the norm for measuring Tre6P in plant tissues. Use of MS/MS has the advantage of greater specificity over single MS due to the additional level of selectivity from fragmentation of the parent ion and detection of multiple, diagnostic product ions (Lunn et al., 2006; Figueroa et al., 2016). Anion-exchange chromatography requires use of an in-line electrolytic suppressor to reduce the ion load from the eluate before it enters the mass spectrometer (Lunn et al., 2006). With careful maintenance, the first generation of electrolytic suppressors could be used for up to 2,000 analytical runs (about 6 months of full-time usage) before needing to be replaced. Suppressor technology has since improved, and, in our experience, current models require little maintenance and provide trouble-free operation for over 4,000 analytical runs. Alternative chromatographic separation methods, such as hydrophilic interaction liquid chromatography, have also be used instead of anion-exchange chromatography for separation of Tre6P from its isomers, but require more complicated sample preparation (Sastre Toraño et al., 2012). LC-MS/MS-based assays allow simultaneous measurements of many other metabolites along with Tre6P, including other phosphorylated intermediates, nucleotide sugars, adenine and nicotinamide cofactors, and organic acids (Lunn et al., 2006; Arrivault et al., 2009, 2015; Figueroa et al., 2016). The range of metabolites that can be measured depends on the type of separation matrix used for the LC phase. Therefore, when setting up an LC-MS/MS system to measure Tre6P, the choice of chromatographic column will often be determined by which other metabolites it is desirable to measure in parallel with Tre6P.

Not all published Tre6P assay methods have been validated as rigorously as the original anion-exchange LC-MS/MS approach (Lunn et al., 2006). The need to use demonstrably pure or enzymatically calibrated Tre6P standards is not always observed, and correction for matrix effects is very difficult unless an isotopically labeled (e.g. $[^2H_2]$Tre6P) internal standard is used for every sample (Lunn et al., 2006; Arrivault et al., 2015). Without these precautions, measurements of Tre6P are prone to significant, but avoidable, technical error.

The presence of other disaccharide-monophosphates in plant extracts is a further potential source of technical error. For example, unless grown axenically, plant samples can contain substantial amounts of α-maltose 1-phosphate derived from bacterial pathogens (Piazza et al., 2015) or other bacterial contaminants. Mass

spectrograms from Arabidopsis extracts typically contain at least four, so far unidentified, peaks with the properties of disaccharide-monophosphates, even when the plants have been grown axenically (Lunn et al., 2014). The presence of known and unknown disaccharide-monophosphates in plant extracts can lead to overestimation of Tre6P, unless the isomeric compounds are demonstrably resolved from Tre6P during the LC phase, or MS/MS is used to differentiate coeluting isomers from their fragmentation patterns. Preincubation of tissue extracts with purified TPP, to check for specific loss of the mass spectrogram peak assigned to Tre6P, is an effective way to demonstrate the specificity of an assay method (Lunn et al., 2006). Although LC-MS/MS is now a well established method for Tre6P analysis, several pitfalls remain to trap the unwary, and the reliability of published Tre6P data should always be assessed against stringent criteria for the specificity and reliability of the extraction and assay methods (Lunn et al., 2006).

THE Suc-Tre6P NEXUS

Following the development of a reliable assay, Tre6P was measured in Arabidopsis plants growing under various experimental conditions, revealing huge fluctuations in the level of Tre6P linked to changes in carbon (C) status. C-starved seedlings (grown in axenic culture) were found to have extremely low levels of Tre6P, but within 15 to 30 min of supplying Suc exogenously, Tre6P began to rise rapidly, eventually reaching levels over 25 times higher than in the C-starved seedlings (Lunn et al., 2006). It was noted that both Suc and Tre6P rose substantially above the levels seen in nonstarved seedlings. In contrast, the other measured metabolites (Glc, Fru, sugar-phosphates, and respiratory intermediates) also increased after Suc addition, but plateaued once they reached the levels of the nonstarved controls. In rosettes of soil-grown Arabidopsis plants, Tre6P also closely tracks the endogenous fluctuations in Suc content during the diurnal light-dark cycle (Lunn et al., 2006; Carillo et al., 2013; Martins et al., 2013). These striking correlations between Tre6P and Suc led to the proposal that Tre6P is a signal of the plant's C status and possibly a specific signal for the availability of Suc (Lunn et al., 2006).

Strong, positive correlations between Suc and Tre6P levels have since been observed in various other Arabidopsis tissues and developmental stages (Wingler et al., 2012; Carillo et al., 2013; Martins et al., 2013; Nunes et al., 2013a; Ragel et al., 2013; Wahl et al., 2013; Sulpice et al., 2014; Yadav et al., 2014), and in other species, including potato (*Solanum tuberosum*; Debast et al., 2011), wheat (*Triticum aestivum*; Martínez-Barajas et al., 2011), maize (*Zea mays*; Henry et al., 2014), and cucumber (*Cucumis sativa*; Zhang et al., 2015). Sugar-addition experiments with C-starved seedlings showed that only Suc, or sugars that can be readily metabolized to Suc (e.g. Glc and Fru), triggered a rapid rise in Tre6P (Yadav et al., 2014). There was no significant change in the level of Tre6P within 3 h of supplying trehalose or sugar analogs that cannot be converted to Suc in vivo. The responsiveness of Tre6P to other major nutrients was investigated in experiments comparing nitrogen (N), phosphorus (P), or sulfur (S) starved seedlings with nonstarved controls. N starvation led to a 3- to 4-fold increase in Tre6P, which was slowly reversed after addition of nitrate or ammonium (Yadav et al., 2014). However, there were concomitant changes in Suc, and Tre6P was highly correlated with Suc across all treatments. This suggested that the apparent response of Tre6P to changes in N status was indirect and likely to be a reflection of the fluctuations in Suc content. There was little or no response of Tre6P to changes in P or S status (Yadav et al., 2014). Together, these results supported the idea that Tre6P is a specific signal of Suc status.

Conceptually, Suc could trigger a rise in Tre6P levels by either stimulating its synthesis by TPS, inhibiting its dephosphorylation by TPP, or both. In C-starved seedlings, the rise in Glc6P and UDPG levels after Suc addition might increase TPS activity if the enzyme were substrate limited in vivo, but Tre6P levels are often poorly correlated with Glc6P or UDPG, arguing against stimulation of TPS activity by a simple, mass-action effect at the substrate level (Yadav et al., 2014). To date, there have been few detailed studies of the kinetics and regulatory properties of higher plant TPS and TPP enzymes, and it is not known if their activities are directly sensitive to Suc. However, inhibitor studies suggested that less direct mechanisms are likely to play an important role in the response of Tre6P to changes in Suc content.

It was shown that the Tre6P response to Suc is insensitive to cordycepin, and so independent of de novo transcription, but is strongly inhibited by cycloheximide, indicating a dependence on de novo protein synthesis (Yadav et al., 2014). This dependence could explain the short (~15 min) lag in the response of Tre6P after Suc addition (Lunn et al., 2006). Polysome-loading analysis showed no significant changes in the ribosomal occupancy (a proxy for translation rate) of any of the *AtTPS* or *AtTPP* gene transcripts, except for a small decrease for *AtTPPJ*, and there was no evidence, from immunoblotting analysis, of changes in the abundance of AtTPS1, the major catalytically active TPS isoform in seedlings (Yadav et al., 2014). Based on these results, it was suggested that increasing Suc might trigger a rise in Tre6P by stimulating synthesis of one or more proteins that activate TPS, or inhibit TPP, or both.

To identify which of the two enzymes, TPS or TPP, is a target for Suc-driven changes in activity, the relationship between Tre6P and Suc was investigated in *35S:otsA* and *35S:otsB* Arabidopsis plants that constitutively express the *E. coli* TPS or TPP, respectively. Unlike the more complex plant enzymes, the *E. coli* TPS and TPP have no obvious structural features beyond their catalytic domains (Lunn, 2007) and seemed likely to be unregulated if expressed in plants. Therefore, overexpression of the bacterial TPS was expected to

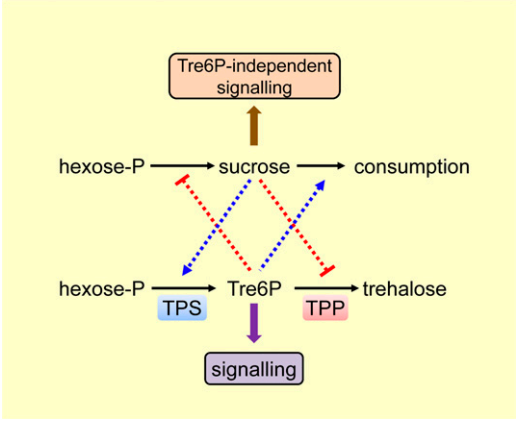

Figure 2. The Suc-Tre6P nexus in plants. The figure represents the core concept of the nexus model, which postulates that Tre6P is both a signal and negative feedback regulator of Suc levels in plants, thereby helping to maintain Suc levels within an optimal range (Yadav et al., 2014). Within the nexus, an increase or decrease in Suc leads to a respective rise or fall in Tre6P levels, via changes in the relative activities of TPS and TPP. In addition to this positive circuit, there is a negative feedback loop by which any increase or decrease in Tre6P leads to an opposite change in Suc levels, via reciprocal effects on Suc production and consumption. There are Tre6P-independent and Tre6P-dependent Suc signaling pathways stemming from the core nexus. This multiplicity of Suc-signaling pathways can give rise to conflicting sugar signals in mutants where imposed changes in Tre6P lead to an opposite and inappropriate change in Suc levels. Blue arrows indicate activation, and red lines indicate inhibition.

outweigh regulation of the endogenous plant TPS and sever the link between Suc and Tre6P if Suc acts mainly on TPS, but not if the plant TPP were the major target for regulation by Suc. Likewise, overexpression of the bacterial TPP was expected to break the Suc-Tre6P relationship if the plant TPP were the main target for Suc. Surprisingly, the correlation between Suc and Tre6P across a range of growth conditions was just as strong in the *35S:otsA* and *35S:otsB* plants as in the wild-type controls, although the ratio of Tre6P:Suc was higher than the wild type in the *35S:otsA* plants and lower than the wild type in the *35S:otsB* plants (Yadav et al., 2014).

This unexpected finding led to the proposal of the Suc-Tre6P nexus model (Fig. 2), which postulates that Tre6P is not only a signal of Suc availability, but also a negative feedback regulator of Suc levels, thereby helping to maintain Suc levels within a range that is optimal for the plant (Yadav et al., 2014). According to this model, the imposed changes in Tre6P, driven by expression of the heterologous TPS and TPP, trigger reciprocal changes in Suc, causing pronounced shifts in the Tre6P:Suc ratio. However, the endogenous mechanisms linking Suc and Tre6P still operate, so the two metabolites remain tightly correlated despite the shift in the Tre6P:Suc ratio. Although mechanistically very different, the proposed control of plant Suc levels by Tre6P in the nexus model can be seen as functionally analogous to the control of blood Glc levels by the insulin-glucagon system in animals (Nelson and Cox, 2013).

Meta-analysis of published Tre6P and Suc data from Arabidopsis indicated that the Tre6P:Suc ratio can be over 10 times higher in sink tissues (e.g. shoot apices) than in source leaves (Lunn et al., 2014). The ratio also varies with the developmental stage of sink organs, such as developing grape (*Vitis vinifera*) berries (Dai et al., 2013) and developing wheat and maize seeds (Martínez-Barajas et al., 2011; Henry et al., 2015). Environmental conditions can also affect the Tre6P:Suc ratio. For example, growth of Arabidopsis plants at 8°C led to a 10-fold accumulation of Suc, compared to plants grown at 20°C, but only a 2-fold increase in Tre6P (Carillo et al., 2013). Nevertheless, the correlation between Suc and Tre6P during the diurnal light-dark cycle was equally strong in both the 20°C and cold-treated plants, despite a 5-fold difference in the Tre6P:Suc ratios. These observations indicated there is flexibility in the nexus, with the target range and sensitivity of the Tre6P-Suc relationship being set according to the particular needs of the tissue and adjusted as the tissue develops, or in response to a change in environmental conditions. In the following sections, we examine how the Suc-Tre6P nexus concept can be used to interpret both the functions of Tre6P in wild-type plants and the effects of manipulating Tre6P levels in specific source and sink tissues, and we describe progress toward understanding the molecular mechanisms that create the nexus.

Tre6P REGULATES PHOTOASSIMILATE PARTITIONING IN LEAVES DURING THE DAY

The primary functions of leaves are to fix CO_2 and supply the rest of the plant with fixed C and energy, which in most vascular plants is achieved by exporting Suc via the phloem (Stitt et al., 2010). During the day, plants set aside a portion of their photoassimilates to accumulate a carbohydrate reserve in the leaves, most commonly in the form of transitory starch (Stitt and Zeeman, 2012). This reserve is remobilized at night to provide substrates for maintenance respiration in the leaf and synthesis of Suc for export to sink organs. One of the contrasting metabolic phenotypes of the constitutive *35S:otsA* and *35S:otsB* plants is a difference in starch content, with the TPS overexpressors having more starch than wild-type plants, while the TPP overexpressors have less, apparently implicating Tre6P in regulation of starch synthesis (Kolbe et al., 2005).

Starch is synthesized from ADP-Glc (ADPG), which is produced by ADPG pyrophosphorylase (AGPase) in the chloroplasts (Stitt and Zeeman, 2012). This enzyme is allosterically activated by 3-phosphoglycerate and inhibited by orthophosphate. It is also subject to redox regulation via reversible formation of a disulphide bridge between the two small subunits in the heterotetrameric enzyme (Tiessen et al., 2002; Hendricks et al., 2003). Immunoblotting analysis showed that AGPase was predominantly in the more active, reduced

(dithiol) form in *35S:otsA* Arabidopsis plants, which have high Tre6P, contrasting with *35S:otsB* plants where AGPase was in the less active, oxidized (disulphide) form (Kolbe et al., 2005). The implication that Tre6P might regulate starch synthesis by modulating the redox status of AGPase appeared to be supported by experiments with isolated pea (*Pisum sativum*) chloroplasts, which showed that incubation with Tre6P and DTT (a strong reducing agent) led to reduction of the AGPase small subunit (Kolbe et al., 2005). However, these experiments were done at a time when the only commercial source of Tre6P (from Sigma-Aldrich) contained only 60 to 70% Tre6P (Lunn et al., 2006). In retrospect, it can be seen that a crucial control treatment was overlooked in the isolated chloroplast experiments of Kolbe et al. (2005), namely, to incubate chloroplasts with Tre6P that had been pretreated with purified TPP to remove the Tre6P and so test for background effects from the contaminants in the Tre6P stock. Later analysis of the Sigma-Aldrich Tre6P available during this early period of plant Tre6P research revealed the presence of over 40 contaminants, including several fatty acids and fatty-acyl esters, other sugar phosphates, and an artificial surfactant (Solutol HS-15; Yadav et al., 2014). If, as seems likely, such detergent-like molecules were present in the isolated chloroplast experiments of Kolbe et al. (2005), they could have disrupted the chloroplast envelope membranes allowing rapid entry of the DTT and reduction of AGPase, independently of any effects of Tre6P itself.

To directly test the hypothesis that Tre6P stimulates starch synthesis by modulating the redox status of AGPase in vivo, Martins et al. (2013) expressed the *otsA* gene under the control of an ethanol-inducible promoter and investigated the effect of induced, short-term increases in Tre6P on starch metabolism. Induction of the ethanol-inducible TPS (iTPS) plants at the beginning of the photoperiod led to a rapid increase in Tre6P levels and a small rise in starch content toward the end of the day but no significant change in the redox status of AGPase at any time (Martins et al., 2013). In similar experiments with the iTPS lines, a very minor stimulation of net starch accumulation was seen in induced plants growing in long-day (16-h photoperiod) conditions, but there was no effect on starch content when the plants were grown in a 12-h photoperiod (Figueroa et al., 2016). Radiolabeling experiments with $^{14}CO_2$ showed no significant effect of Tre6P on C flux into starch (Figueroa et al., 2016). Thus, it was concluded that Tre6P has no direct influence on the redox status of AGPase in leaves and only a marginal influence on net transitory starch accumulation during the day. Furthermore, the role of redox modulation of AGPase in control of transitory starch synthesis has been called into question anyway because abolishing the potential for redox regulation (by site-directed mutagenesis of AGPase) had little or no effect on net rates of starch accumulation, except under very C-limiting (short days and low light) conditions (Li et al., 2012; Hädrich et al., 2012; Mugford et al., 2014).

One of the predictions of the nexus model is that an artificially induced rise in Tre6P should lead to a drop in Suc, and this was indeed observed after induction of otsA expression in Arabidopsis leaves (Figueroa et al., 2016). At the same time, there were significant increases in the levels of tricarboxylic acid pathway intermediates (citrate, aconitate, 2-oxoglutarate, fumarate, and malate) and several amino acids. Stable ($^{13}CO_2$) and radio-isotope ($^{14}CO_2$) labeling experiments showed lower flux of newly fixed C into Suc, accounting for the overall drop in Suc levels (Figueroa et al., 2016). In contrast, there was increased anaplerotic flux of C into organic acids in the induced plants, associated with increased phosphorylation of phospho*enol*pyruvate (PEP) carboxylase (PEPC) on Ser-11 and decreased monoubiquitination of the enzyme. Both of these changes in covalent modifications activate PEPC by increasing the enzyme's affinity for its substrate, PEP, and allosteric activator, Glc6P, and decreasing its sensitivity to inhibition by malate (O'Leary et al., 2011). In parallel, high Tre6P also triggered a decrease in phosphorylation of nitrate reductase (NR) on Ser-534, thereby activating the enzyme (Figueroa et al., 2016). NR catalyzes the first step of nitrate assimilation, the reduction of nitrate to nitrite in the cytosol, which is rapidly followed by reduction of nitrite to ammonium in the chloroplasts (Su et al., 1996; Kaiser and Huber, 2001). Thus, the simultaneous activation of PEPC and NR could account for the Tre6P-induced rise in amino acid levels, by stimulating nitrate assimilation into ammonium as well as the production of C-skeletons needed for their synthesis (Fig. 3A).

There was evidence of increased citrate synthesis in the induced plants, implying increased activity of the mitochondrial pyruvate dehydrogenase (mPDH) as well as PEPC and NR (Figueroa et al., 2016). Potentially this could be brought about by Tre6P-triggered changes in the phosphorylation status of the mPDH. This enzyme is usually inactivated by phosphorylation during the day (Budde and Randall, 1990; Gemel and Randall, 1992; Tovar-Méndez et al., 2003), restricting the provision of acetyl-CoA for citrate synthesis, which mainly occurs during the night (Gauthier et al., 2010; Tcherkez et al., 2012; Ishihara et al., 2015).

As an aside, a correlative relationship between Suc, Tre6P, and the posttranslational activation status of PEPC has also recently been reported in cluster roots of white lupin (*Lupinus albus*), which synthesizes organic acids in the roots and exudes them into the rhizosphere to mobilize Pi, iron, and other nutrients for uptake by the roots (Shane et al., 2016). White lupin root exudates can represent up to 25% of the plant's overall C budget, and it was suggested that Tre6P links the activation status of PEPC and anaplerotic synthesis of organic acids in the roots to the supply of Suc from the source leaves.

It is not yet known how Tre6P brings about the changes in activation status of PEPC and NR and whether it acts directly or indirectly on the enzymes that modify PEPC and NR. PEPC is phosphorylated by

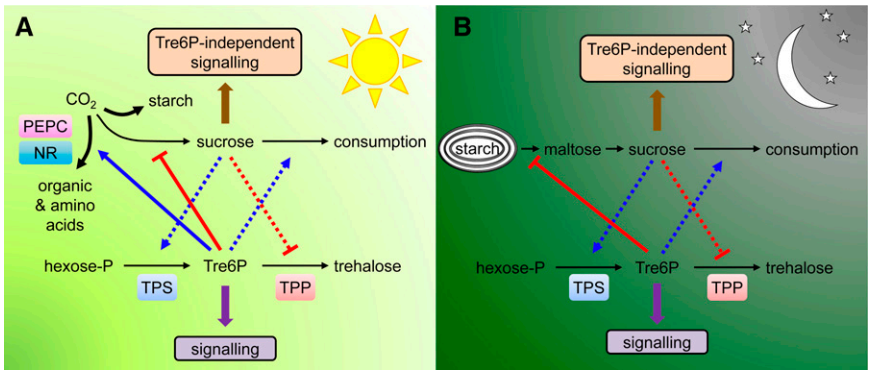

Figure 3. The Suc-Tre6P nexus in source leaves. It is proposed that the core Suc-Tre6P nexus and the associated signaling pathways are adapted to meet the particular needs of individual tissues. In illuminated source leaves (A), Tre6P regulates the partitioning of photoassimilates between Suc and organic and amino acids by influencing the posttranslational regulatory status of PEPC and NR. In source leaves at night (B), Tre6P regulates transitory starch breakdown, balancing the supply of substrates for Suc synthesis with the demand for Suc from sink organs. Blue arrows indicate activation, and red lines indicate inhibition. Solid lines show experimentally demonstrated interactions, while dashed lines represent hypothetical interactions.

a specific PEPC kinase (Hartwell et al., 1999), and NR can be phosphorylated by either SUC-NON-FERMENTING-1-RELATED KINASE1 (SnRK1) or calcium-dependent protein kinases (Bachmann et al., 1995, 1996; Sugden et al., 1999). The corresponding protein phosphatases and the molecular machinery involved in mono-ubiquitination of PEPC are less well characterized. Inhibition of SnRK1 by Tre6P (Zhang et al., 2009) could, in principle, account for the activation of NR. However, inhibition of SnRK1 by Tre6P appears to be restricted to developing tissues (Zhang et al., 2009), being dependent on an uncharacterized protein factor that is absent from source leaves where activation of NR by Tre6P was observed by Figueroa et al. (2016). There was also no evidence of SnRK1-mediated changes in the activation status of Suc-phosphate synthase, a classical SnRK1 target protein (Huber and Huber, 1996), in response to high Tre6P (Figueroa et al., 2016). Thus, further studies are needed to understand how rising Tre6P levels lead to activation of PEPC and NR and how the deduced activation of mPDH is brought about (see Outstanding Questions).

These results can be interpreted in the context of source-sink relations. If Suc synthesis in source leaves exceeds sink demand, Suc will tend to accumulate in the leaves triggering a rise in Tre6P. In turn, this will activate PEPC and NR, diverting photoassimilates into organic and amino acids and away from Suc synthesis, thereby bringing the level of Suc back down again. Sink limitation can arise if there is insufficient synthesis and export of amino acids from the leaves. In such a scenario, the diversion of photoassimilates into organic and amino acids, triggered by rising Tre6P, will not only bring Suc levels back into balance, but also help to alleviate the sink limitation by stimulating the supply of amino acids from the leaves. Thus, the recent findings of Figueroa et al. (2016) directly implicate Tre6P in coordination of C and N metabolism and in source-sink relations. While this might appear to be a specific function for Tre6P in leaves during the day, it is essentially consistent with the Suc-Tre6P nexus concept, i.e. that the fundamental function of Tre6P is to signal and regulate Suc levels. As with many other signal molecules, the targets and molecular mechanisms by which Tre6P exerts its primary function can differ between tissues and are adapted to meet the specific needs of a particular tissue. This idea will be a recurring theme in the following sections.

Tre6P REGULATES TRANSITORY STARCH DEGRADATION IN LEAVES AT NIGHT

Although current evidence indicates that Tre6P plays little role in regulating starch synthesis in Arabidopsis leaves during the day, it clearly does have an impact on starch degradation at night (Martins et al., 2013; Figueroa et al., 2016). In the dark, transitory starch reserves are degraded by β-amylase (BAM) and debranching enzymes, yielding maltose and Glc, which are exported from the chloroplasts to provide substrates for respiration and Suc synthesis (Stitt and Zeeman, 2012). In Arabidopsis plants growing under C-limiting conditions (e.g. short days or low light), almost all of the transitory starch reserve is degraded during the night, leaving just a small residue at dawn (Graf et al., 2010). The rate of starch degradation is carefully controlled so that the plant can make maximal use of its carbohydrate reserves but avoid running out of starch before dawn, when photosynthesis can resume. The circadian clock plays a key role in setting the rate of starch degradation under these conditions (Graf et al., 2010). As dusk falls, the plant somehow senses the amount of starch it has accumulated during the day and anticipates the length of the coming night period using outputs from the clock and then combines this information to set an appropriate rate of degradation (Scialdone et al., 2013).

Experiments with the iTPS Arabidopsis lines revealed that high nighttime levels of Tre6P inhibit starch degradation, leading to a starch excess phenotype at the end of the night (Martins et al., 2013). Given that Tre6P mirrors leaf Suc levels at night, as well as during the day (Carillo et al., 2013), it was proposed that inhibition by Tre6P links the rate of starch degradation to the level of Suc in the leaf and ultimately to Suc demand from sink organs (Martins et al., 2013). It was envisaged that the circadian clock sets an upper limit for the permissible rate of starch degradation to prevent the plant from running out of starch before dawn, but the actual rate of starch degradation is adjustable, via the Suc-Tre6P negative feedback circuit, to balance the supply and demand for Suc (Fig. 3B). For example, if sink demand was low (e.g. due to low nighttime temperatures), Suc would accumulate in the leaves, triggering a rise in Tre6P and inhibition of starch degradation. In this way, the starch reserves would not be remobilized unless needed, and wasteful respiration of excess sugars in the leaves would be avoided. Conversely, if sink demand for Suc exceeds the current supply, leaf Suc levels, and thus Tre6P, would fall, allowing starch degradation to accelerate up to the maximal rate set by the clock and so increase the supply of Suc.

The molecular mechanism by which Tre6P inhibits transitory starch degradation is not yet known. The induced iTPS Arabidopsis plants with high nighttime levels of Tre6P contained less maltose and Suc than noninduced control plants, indicating that an early step in the pathway of starch degradation within the chloroplasts was affected (Martins et al., 2013). The first step in the pathway involves phosphorylation of the glucan chains at the surface of the starch granule by glucan, water dikinase (GWD) and phosphoglucan, water dikinase (PWD; Baunsgaard et al., 2005; Ritte et al., 2002, 2006; Kötting et al., 2005). This opens up the α-helical structure of the glucan chains, allowing access to the starch degrading enzymes (Edner et al., 2007). Dephosphorylation of the glucan chains by two phosphoglucan phosphatases (SEX4 and LSF2) is also necessary for complete degradation of starch (Niittylä et al., 2006; Kötting et al., 2009; Hejazi et al., 2010; Santelia et al., 2011). Starch granules extracted from induced iTPS plants with high levels of Tre6P had more phosphate than granules from noninduced control plants (Martins et al., 2013). This suggested that Tre6P interferes with the cycle of starch phosphorylation and dephosphorylation, although inhibition of BAM could not be excluded as a possibility (Martins et al., 2013). In vitro assays of recombinant GWD, PWD, SEX4, and BAM3 (the major BAM isoform involved in starch breakdown in mesophyll cells) showed no evidence of direct activation or inhibition by Tre6P (or trehalose), and there were no obvious changes in the abundance of these enzymes in the induced plants with high Tre6P (Martins et al., 2013).

It is also unclear whether Tre6P inhibits starch degradation by acting directly in the chloroplasts or indirectly via some intermediary in the cytosol or at the chloroplast surface. AtTPS1 is a predominantly cytosolic enzyme (Vandesteene et al., 2010), and its UDPG substrate is essentially restricted to the cytosol (Szecowka et al., 2013). Therefore, Tre6P is thought to be made primarily in the cytosol, and nonaqueous fractionation showed that the vast majority of the Tre6P in leaf cells is located outside the chloroplasts and vacuoles (i.e. most likely in the cytosol; Martins et al., 2013). The small amount of Tre6P assigned to the chloroplasts by the nonaqueous fractionation analysis was within the range of the technical error, raising doubts whether any Tre6P is present in the chloroplasts or not. However, the recently confirmed presence of at least one isoform of TPP (AtTPPD) in chloroplasts implies that Tre6P does enter the chloroplasts (Krasensky et al., 2014). Plastidial TPP activity might also explain why the concentration of Tre6P in the chloroplasts is very low and close to the limits of detection with current cell fractionation methods. To identify the target(s), location, and mode of action of Tre6P, we initiated a forward genetics screen for suppressor mutations that abolish the starch excess phenotype in the inducible TPS background. To date, 11 putative *loss of starch excess* mutants have been identified, and mapping of the causal genetic lesions is underway. At this point, it is worth noting that inhibition of starch degradation by Tre6P, rather than stimulation of starch synthesis, probably accounts for the high starch content of the constitutive *35S:otsA* plants observed by Kolbe et al. (2005).

Interpreting these results in the context of the nexus model, the inhibition of starch degradation by Tre6P at night can be seen as another adaptation of the fundamental Suc-Tre6P relationship, in this instance to meet the particular needs of leaves in the dark. It is also interesting to note that both the daytime and nighttime feedback circuits for regulating Suc levels in source leaves operate by inhibiting Suc synthesis, rather than stimulating Suc consumption (Fig. 3, A and B), although further work might reveal effects of Tre6P on Suc utilization in leaves as well as its production.

Tre6P, SnRK1, AND THE CONTROL OF GROWTH IN DEVELOPING SINK ORGANS

The growth of heterotrophic tissues, such as meristems, roots, flowers, and developing seeds, depends on supplies of photoassimilates from the leaves or on remobilization of starch or other storage reserves (e.g. in germinating seeds or sprouting tubers; Fig. 4). Suc is the most common form in which carbon is transported from source tissues via the phloem. When it arrives in sink tissues, Suc is unloaded from the phloem and cleaved by acid invertases in the cell wall and vacuoles or by Suc synthase and neutral/alkaline invertases in the cytosol, chloroplasts, and mitochondria (Barratt et al., 2009; Lunn, 2016). The resulting hexoses and hexose-phosphates can then enter central metabolism and be used for synthesis of cell walls, storage polysaccharides, proteins, lipids, and nucleic acids or respired to provide energy and reducing equivalents. Thus, Suc is the main input into C metabolism in sink

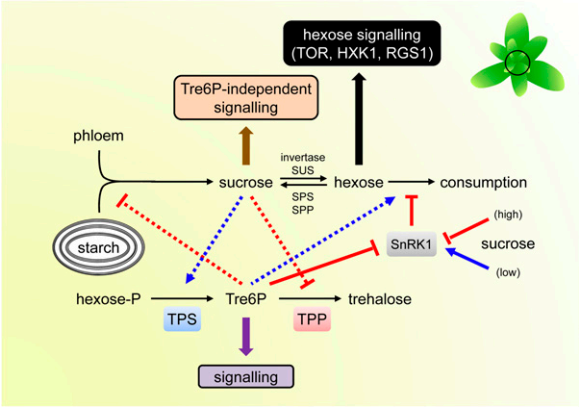

Figure 4. The Suc-Tre6P nexus in sink organs. Growing sink tissues import Suc, which is catabolized by invertases and Suc synthase (SUS) to provide carbon and energy for growth and accumulation of storage reserves. Resynthesis of Suc via Suc-phosphate synthase (SPS) and Suc-phosphate phosphatase (SPP) can occur in parallel with net Suc catabolism. Tre6P regulates consumption of Suc in Suc-importing sink organs (e.g. meristems and developing seeds), mediated in part by inhibition of SnRK1. SnRK1 is activated by low energy status, so Tre6P might also influence SnRK1 activity indirectly via effects on Suc and energy levels. It is likely that Suc consumption is also regulated in ways that are not directly dependent on SnRK1. By analogy with source leaves, Tre6P might regulate turnover of transitory starch reserves in sink organs. Any changes in hexose levels or the Suc:hexose ratio are likely to trigger other sugar signaling responses mediated by TOR, hexokinase1 (HXK1), or REGULATOR OF G-PROTEIN SIGNALING1 (RGS1). Blue arrows indicate activation, and red lines indicate inhibition. Solid lines show experimentally demonstrated interactions, while dashed lines represent hypothetical interactions.

organs and Suc availability is a major determinant of growth.

Two evolutionarily conserved protein kinases, SnRK1 and TARGET OF RAPAMYCIN (TOR), play central (and antagonistic) roles in regulating growth by integrating information about nutrient status and environmental conditions (Baena-González et al., 2007; Emanuelle et al., 2015; Dobrenel et al., 2013, 2016). One of these control hubs, SnRK1, is potentially regulated by Tre6P (Zhang et al., 2009; Delatte et al., 2011). No direct link between Tre6P and TOR signaling has yet been described in plants. SnRK1, like its homologs in yeast (Suc-Non-Fermenting 1 [SNF1]) and mammals (AMP-activated protein kinase), has a highly conserved function in energy sensing and homeostasis and is also involved in abiotic stress responses in plants (Baena-González et al., 2007). SnRK1 is activated when energy levels are low, leading to inhibition of energy-consuming biosynthetic processes and stimulation of energy-producing catabolic processes. Although the importance of SnRK1 in regulation of growth is not in doubt (Radchuk et al., 2010), there is some uncertainty about the precise nature of its relationship with Tre6P. This topic was discussed at length in a recent review by Lunn et al. (2014), so here we shall recap only the main points.

SnRK1 activity in crude extracts from developing Arabidopsis tissues is inhibited by Tre6P with a K_i of 5 μM (Zhang et al., 2009; Nunes et al., 2013b), which lies within the estimated range (4–7 μM) of in vivo Tre6P concentrations in Arabidopsis rosettes in the light (Martins et al., 2013). However, there is some uncertainty about the purity of the Tre6P used for some of the in vitro experiments. As noted above, pre-2011 stocks of Tre6P from Sigma-Aldrich, the only commercial source at the time, contained only 60 to 70% Tre6P and at least 40 identifiable contaminants (Lunn et al., 2006, Yadav et al., 2014). SnRK1 is also inhibited by Glc 1-phosphate (Glc1P) and Glc6P, with K_i values of 55 and 300 μM, respectively (Nunes et al., 2013b). It is not known whether the sensitivity of SnRK1 to Tre6P is affected by the presence of these other inhibitors. SnRK1 that had been partially purified by immunoprecipitation was not inhibited by Tre6P, but sensitivity to Tre6P was restored by adding back the supernatant from the immunoprecipitation, indicating that some other factor was needed (Zhang et al., 2009). This factor is thought to be a protein that is only present in developing tissues and has not yet been unidentified.

Experimental evidence for inhibition of SnRK1 by Tre6P in vivo came initially from comparison of gene expression patterns in *35S:otsA* Arabidopsis plants, with constitutively high levels of Tre6P, and wild-type plants (Zhang et al., 2009; Paul et al., 2010; Wingler et al., 2012). About 50% of the genes that were up- or down-regulated in the *35S:otsA* plants showed the opposite response when KIN10 (a catalytically active α-subunit of the Arabidopsis SnRK1) was transiently expressed in Arabidopsis mesophyll protoplasts (Baena-González et al., 2007; Zhang et al., 2009). In a follow-up analysis, expression of a subset of SnRK1 reporter genes was seen to be correlated with Tre6P in Arabidopsis seedlings subjected to various treatments that alter Tre6P levels (Nunes et al., 2013a). In potato tubers, about 20% of the genes that were differentially expressed following tuber-specific overexpression of TPP also overlapped with the KIN10-responsive genes in Arabidopsis protoplasts (Debast et al., 2011). From these findings, there emerged a model that Tre6P (reflecting Suc levels) promotes growth by inhibiting SnRK1 activity in sink tissues (Zhang et al., 2009; Paul et al., 2010; Smeekens et al., 2010; Nunes et al., 2013c; O'Hara et al., 2013). However, other findings point to a more complex relationship between Tre6P and SnRK1, and are difficult to reconcile with this simple, linear model.

A recent transcriptomic analysis of progressive C starvation and responses to Suc readdition in Arabidopsis seedlings revealed a complex picture of transcriptional responses to C status (Cookson et al., 2016). When compared with published data sets, there was only a partial overlap between the Suc- and KIN10-responsive genes and only a partial overlap between these two sets of genes and those genes that were differentially expressed in *35S:otsA* plants (versus the wild type). In the early stages of C starvation, the changes in

gene expression suggested a major role for the bZIP11 transcription factor (Cookson et al., 2016), which is translationally regulated by Suc via ribosomal binding to an upstream open reading frame in the *bZIP11* transcript (Wiese et al., 2005; Hanson et al., 2008; Rahmani et al., 2009; Ma et al., 2011; Weiste et al., 2014). One of the main conclusions from the transcriptomic analysis of Cookson et al. (2016) is that Suc affects gene expression at the transcript level via multiple routes, and signaling via SnRK1 accounts for only part of the transcriptional response to changes in C status.

This scenario complicates interpretation of the differential gene expression in *35S:otsA* compared to wild-type plants (Zhang et al., 2009) because Tre6P is not the only metabolite affected in these plants; along with elevated Tre6P, *35S:otsA* plants also have constitutively decreased Suc levels (Yadav et al., 2014). As a result, constitutive TPS-overexpressing plants are likely to be experiencing "high-sugar" signals from the elevated Tre6P and, at the same time, "low-sugar" signals from Suc itself (via Tre6P-independent sugar signaling pathways). Consequently, these plants are constantly receiving mixed signals about their sugar status. It was also observed that constitutive *35S:otsA* plants grown in 8-, 12-, or 16-h photoperiod conditions have lower relative growth rates than wild-type plants (Yadav et al., 2014). This appears to be at odds with the idea that high Tre6P promotes growth via inhibition of SnRK1 (Zhang et al., 2009), which predicts that *35S:otsA* plants should grow faster, not slower, than wild-type plants. It is possible that developmental changes triggered by the high Tre6P, such as precocious flowering (Schluepmann et al., 2003; Wahl et al., 2013), have a confounding effect on rosette growth by diverting resources to reproductive rather than vegetative growth. However, another explanation for the reduced growth rate of *35S:otsA* plants is simply that they have low Suc levels, triggering "low-sugar/low-energy" responses, including SnRK1, that restrict growth.

This scenario fits well with the Suc-Tre6P nexus concept, which envisages both Tre6P-dependent and Tre6P-independent signaling events emanating from the core relationship between Suc and Tre6P. According to the nexus model, the constitutive overproduction of Tre6P in *35S:otsA* plants will trigger Tre6P-dependent signaling pathways (e.g. early flowering), but at the same time drive Suc levels down via the negative feedback circuit, and so also trigger "low-sugar/low-energy" signaling pathways (e.g. SnRK1). Further convolutions can be expected from additional side effects of lowered Suc levels on the circadian clock (Haydon et al., 2013), phytohormones (Lilley et al., 2012; Sairanen et al., 2012), and micro-RNA156 signaling (Yang et al., 2013; Yu et al., 2013). Glc and Fru levels are also likely to be affected (Bihmidine et al., 2013), which will have an impact on hexose-sugar signaling pathways mediated by hexokinase (Moore et al., 2003), TOR (Dobrenel et al., 2013, 2016; Xiong et al., 2013), or REGULATOR OF G-PROTEIN SIGNALING1 (Fu et al., 2014). It should also be noted that imposed changes in expression of SnRK1 can affect Tre6P and Suc levels, with both of these metabolites being increased when SnRK1 expression was suppressed in developing pea seeds (Radchuk et al., 2010).

The take-home message is that constitutive *35S:otsA* plants, while providing a dramatic demonstration of the impact of Tre6P on plant growth and development, have limited use for investigating the molecular components of Tre6P signaling due to the confounding effects of simultaneously having high Tre6P but low Suc. Inducible changes in TPS (or TPP) expression offer a potentially more informative experimental system, allowing us to observe responses to increased or decreased Tre6P in an effectively wild-type background and to follow the kinetics of the responses to distinguish primary from secondary effects (Martins et al., 2013; Figueroa et al., 2016). To fully understand the connection between Tre6P and SnRK1 (see Outstanding Questions), it will also be desirable to compare Tre6P and SnRK1 responses in the same tissues and to monitor more direct outputs of SnRK1 activity (i.e. changes in target protein phosphorylation status) rather than transcript levels, which only indirectly reflect SnRK1 activity and, if open to influence by other factors, might not be truly specific reporters of SnRK1 activity.

A REAPPRAISAL OF THE ROLE OF Tre6P AND AtTPS1 IN DEVELOPING ARABIDOPSIS SEEDS

The arrest of Arabidopsis *tps1* embryos at the torpedo stage (Eastmond et al., 2002) is one of the classic examples used to demonstrate the importance of trehalose metabolism, and by implication, Tre6P in plants. However, new information about the TPS family in Arabidopsis raises questions about the function of AtTPS1 in developing seeds, and whether this is limited to synthesis of Tre6P (Delorge et al., 2015). The plant TPS family is split into two distinct and ancient clades, known as class I and class II (Leyman et al., 2001). Class I (e.g. AtTPS1) includes the only isoforms that have been shown to have TPS activity in vitro. They are also the only plant isoforms that are reproducibly able to complement the yeast *tps1Δ* mutant, which lacks Tre6P and is unable to grow on Glc-containing media due to uncontrolled entry of Glc into glycolysis via hexokinase (Blázquez et al., 1998; Van Dijck et al., 2002; Vandesteene et al., 2010; Delorge et al., 2015). The class II TPSs (e.g. AtTPS5-AtTPS11) do not reproducibly complement the yeast *tps1Δ* mutant, despite having a glycosyltransferase domain similar to the catalytic domain of the class I TPSs and almost complete conservation of active site residues (Harthill et al., 2006; Lunn, 2007; Ramon et al., 2009). AtTPS5 has been implicated in thermotolerance (Suzuki et al., 2008) and AtTPS11 in resistance to the aphid pest *Myzus persicae* (Singh et al., 2011), but for the most part the functions of the class II TPSs remain enigmatic.

Apart from some paleopolyploid species, such as maize, diploid plants tend to have a single class I isoform (Lunn, 2007). Therefore, Arabidopsis is unusual in

having four class I genes, *AtTPS1*, *AtTPS2*, *AtTPS3*, and *AtTPS4*, although *AtTPS3* is most likely a pseudogene (Lunn, 2007; Vandesteene et al., 2010). In common with most other class I TPSs, the AtTPS1 protein has a multidomain structure, comprising an N-terminal autoinhibitory domain, the catalytic glycosyltransferase domain, and a C-terminal TPP-like domain of unknown function (Van Dijck et al., 2002). In comparison, AtTPS2 and AtTPS4 are smaller because they lack an N-terminal autoinhibitory domain (Lunn, 2007). Syntenic and similarly truncated isoforms are present in *Arabidopsis lyrata*, *Capsella rubella*, *Brassica rapa*, and other members of the Brassicaceae but appear to be absent from the closely related Caricaceae (papaya) family within the order Brassicales (Ming et al., 2008) and have not been identified in any other plant families (Lunn, 2007). Despite the high conservation of TPS active-site residues in AtTPS2 and AtTPS4, neither was able to complement the yeast *tps1Δ* mutant, suggesting that they lacked TPS activity (Vandesteene et al., 2010). However, it was recently demonstrated, that AtTPS2 and AtTPS4 do complement a yeast *tps1Δ tps2Δ* double mutant, which lacks both TPS and TPP activity, and that the complemented yeast cells contain Tre6P (Delorge et al., 2015). Indeed, the AtTPS2- and AtTPS4-complemented cells accumulated up to 60 times more Tre6P than those complemented by AtTPS1, suggesting that the two smaller isoforms have considerably higher activity than AtTPS1 (Delorge et al., 2015), as might be expected from their lack of an autoinhibitory domain.

The *AtTPS2* and *AtTPS4* genes are expressed almost exclusively in developing seeds (Schmid et al., 2005, Vandesteene et al., 2010) but are apparently unable to substitute for AtTPS1 in developing seeds of the *tps1* mutant (Eastmond et al., 2002), despite being perfectly capable of producing Tre6P (Delorge et al., 2015). Differential expression patterns within the various tissues of the developing seed offer one plausible explanation why AtTPS1 is nonredundant. At the earliest (preglobular) stage of development, *AtTPS1* is expressed in the chalazal seed coat, and then at intermediate (globular and heart) stages in the chalazal endosperm, peripheral endosperm, and the embryo itself, whereas *AtTPS2* and *AtTPS4* expression is restricted to the chalazal and peripheral endosperms, respectively (Fig. 5; Le et al., 2010). However, at the later, torpedo stage (when *tps1* embryos arrest), *AtTPS1* continues to be expressed in the embryo and chalazal tissues and is particularly highly expressed in the peripheral endosperm (Fig. 5). Both *AtTPS2* and *AtTPS4* are also expressed in the peripheral and chalazal (*AtTPS4* only) endosperms at the torpedo stage. Although we do not know for sure that the *AtTPS* transcripts are equally translated into the corresponding proteins, the partial overlap in the expression domains of *AtTPS1*, *AtTPS2*, and *AtTPS4* in the peripheral and chalazal endosperms suggests that some capacity to synthesize Tre6P will be present in these tissues even in the *tps1* mutant. Therefore, we can surmise that either loss of AtTPS1 in the embryo proper is the critical defect leading to embryo arrest in the *tps1* mutant or that AtTPS1 has an essential function in the peripheral or chalazal endosperms that cannot be substituted by AtTPS2 or AtTPS4, i.e. a function apart from its capacity to synthesize Tre6P.

It is intriguing to note that AtTPS1 was found to exist in a high-M_r complex with tubulin, a cell cycle kinase (CDKA;1) and the CDKA;1-interacting kinesin, KCA1, in inflorescence tissues (Geelen et al., 2007), suggesting a role for AtTPS1 in regulation of the cell cycle via direct interactions with other proteins. The possibility that AtTPS1 has noncatalytic functions should therefore be considered when interpreting the phenotypes of Arabidopsis *tps1* mutants and any orthologous mutants in other plants. The physiological significance of the truncated class I isoforms in the Brassicaceae is unclear. Their lack of an autoinhibitory domain appears to give them a high activity (Delorge et al., 2015), so maybe their primary function is simply to produce trehalose, which could contribute to osmoregulation or act as a temporary carbon reserve in the seed endosperm tissues.

The late-flowering phenotype of rescued *tps1* mutants (van Dijken et al., 2004; Gómez et al., 2010) when grown under long-day conditions is due to lack of expression of *FLOWERING LOCUS T* (*FT*), which encodes a phloem-mobile protein that is transported from the leaves to the shoot apical meristem to trigger flowering (Wahl et al., 2013). Curiously, FT is also involved in signaling between maternal fruit tissues and developing seeds. It integrates the thermal history of the mother plant and transmits this information, via the fruit phloem, to affect proanthocyanidin synthesis in the seed coat, which in turn affects seed dormancy (Chen et al., 2014). If *FT* expression in fruits were also dependent on having a functional AtTPS1, as in leaves, then AtTPS1 (and presumably Tre6P) expressed in fruit tissues would have influence over seed dormancy. Impairment of trehalose metabolism in several Arabidopsis mutants (*tps1*, *tppg*, and *tre1*) abolishes the inhibition of seed germination by abscisic acid (ABA), although it is unclear if this is an inherited maternal effect (potentially mediated by FT) or an inherent trait of the mutant seeds (Gómez et al., 2010; Vandesteene et al., 2012; Van Houtte et al., 2013).

To sum up, the enzymes and intermediates of trehalose metabolism, including Tre6P, appear to have multiple functions in seed and fruit development, and loss of noncatalytic functions of AtTPS1 might play some part in the growth arrest of *tps1* embryos at the torpedo stage, in addition to the partial loss of Tre6P-synthesizing capacity.

Tre6P AND ABIOTIC STRESS TOLERANCE

In nature, plants face many challenges from their environment; they must fight off attacks from insects and other herbivores, defend themselves from infection by pathogenic bacteria and fungi, and cope with a whole host of abiotic stresses: drought, heat, cold, salinity,

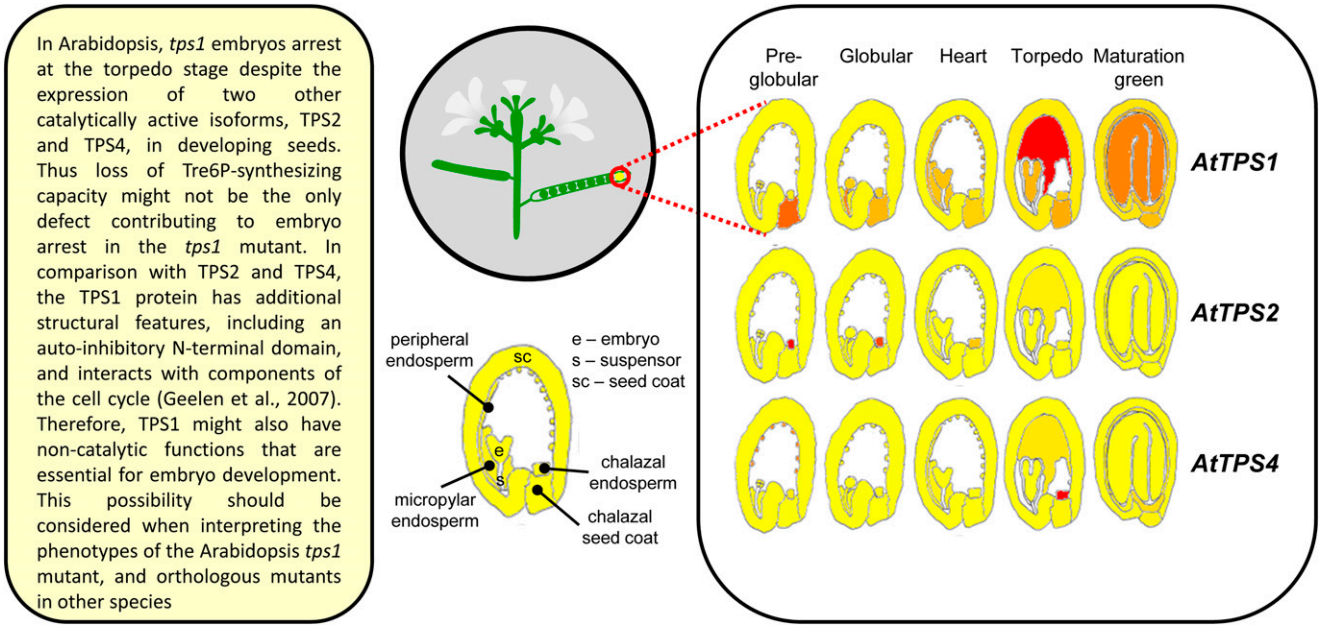

Figure 5. Is synthesis of Tre6P the only function of Arabidopsis TPS1? Arabidopsis has 11 *TPS* genes, divided into two distinct clades: class I (*AtTPS1–AtTPS4*) and class II (*AtTPS5–AtTPS11*). Three of the class I genes, *AtTPS1*, *AtTPS2*, and *AtTPS4*, encode catalytically active Tre6P synthases (*AtTPS3* is most likely a pseudogene). The class II genes encode TPS-like proteins with no apparent Tre6P synthase activity and their functions are largely unknown. The figure shows the spatio-temporal expression patterns of class I *TPS* genes in developing Arabidopsis seeds, based on data from Le et al. (2010) and visualized using the BAR eGFP browser (http://bar.utoronto.ca/; Winter et al., 2007). Arabidopsis *tps1* null mutants arrest at the torpedo stage of embryogenesis, even though the expression of *AtTPS2* and *AtTPS4* partially overlaps with *AtTPS1* expression in the peripheral and chalazal endosperms at this stage in development. This implies that *tps1* mutant seeds retain some capacity to synthesize Tre6P, raising the possibility that loss of noncatalytic functions of AtTPS1 contributes to the defective phenotype of *tps1* mutants.

flooding, high light, UV light, etc. Trehalose is an essential virulence factor for the rice blast fungus, *Magnaporthe oryzae* (Foster et al., 2003; Wilson et al., 2007), and for some bacterial pathogens, including *Pseudomonas* spp. and *Xanthomonas citri* subsp. *citri* (Freeman et al., 2010; Djonović et al., 2013; Piazza et al., 2015), but is a double-edged sword for the pathogens as it is also a trigger for defense responses by the host plant (Reignault et al., 2001; Renard-Merlier et al., 2007; Tayeh et al., 2014; Piazza et al., 2015). Trehalose is also implicated as a signal molecule during formation of rhizobial and mycorrhizal symbioses (Fig. 1) and in interactions with other beneficial microbes (Suárez et al., 2008; Rodríguez-Salazar et al., 2009; Fernandez et al., 2010, 2012). As these topics have been covered in a recent review (Lunn et al., 2014), and no specific function for Tre6P in plant-microbe interactions (beyond the synthesis of trehalose) has so far been clearly identified, we shall focus here on the role of Tre6P in plant responses to abiotic stress.

As nonreducing disaccharides, trehalose and Suc are chemically less reactive than most other naturally occurring sugars and so can be accumulated to high concentrations within cells without damaging cellular structures. Indeed, they both have a stabilizing effect on proteins and membranes in vitro, particularly trehalose, which finds practical application as a stabilizer for vaccines and other labile pharmaceuticals, allowing their storage and transport without refrigeration. Desiccation-tolerant resurrection plants accumulate massive amounts of trehalose or Suc when exposed to drought, and their cells remain viable despite losing ≥95% of their water content (Drennan et al., 1993; Iturriaga et al., 2000; Farrant et al., 2015). One way in which trehalose protects cells is by initiating controlled autophagy, thereby avoiding uncontrolled damage that would otherwise trigger programmed cell death (Williams et al., 2015). Trehalose also confers desiccation tolerance on yeast cells (Tapia et al., 2015), in part by protecting them from oxidative damage by reactive oxygen species (Benaroudj et al., 2001). Many of the abiotic (and biotic) stresses faced by plants lead to reactive oxygen species production, and the accumulation of sugars such as trehalose and Suc is one of the mechanisms providing protection from oxidative stress (Romero et al., 2002; Cortina and Culianez-Macia, 2005; Couée et al., 2006; Luo et al., 2008; Chen and Hoehenwarter, 2015).

In light of its protective capacity in resurrection plants and other organisms, trehalose metabolism was an obvious target for genetic manipulation to improve stress tolerance in plants. Constitutive expression of microbial *TPS* or *TPP* genes can lead to improved stress tolerance, but usually at the expense of undesirable side

effects on growth (Goddijn et al., 1997; Romero et al., 1997; Pilon-Smits et al., 1998; Goddijn and van Dun, 1999). More targeted interventions have generally been more successful strategies; these include (1) limiting expression to specific subcellular compartments or tissues, (2) use of endogenous plant genes, and (3) placing the introduced genes under the control of stress-inducible promoters (Garg, et al., 2002; Lee et al., 2003; Avonce et al., 2004; Miranda, et al., 2007; Stiller et al., 2008; Li et al., 2011; Kondrák et al., 2012). However, in most cases the enhanced stress tolerance did not appear to be linked to increased trehalose accumulation. Indeed, the opposite was true for Arabidopsis *tre1* mutants that lacked trehalase activity and had moderately elevated trehalose levels but were more sensitive to drought than wild-type plants (Van Houtte et al., 2013). Thus, we need to search for alternative explanations for the beneficial effects of manipulating trehalose metabolism on stress tolerance and for understanding the role of trehalose metabolism in endogenous responses to abiotic stresses.

Exposure of plants to low oxygen leads to a widespread reconfiguration of gene expression and metabolism (Bailey-Serres et al., 2012), including a decrease in Tre6P levels in developing Arabidopsis seeds (Thiel et al., 2011). Recently, a quantitative trait locus for anaerobic germination tolerance in rice (*Oryza sativa*) was found to be linked to expression of a functional *OsTPP7* gene in the tolerant genotype, Khao Hlan On (KHO); the *OsTPP7* gene is essentially missing from the intolerant IR64 genotype (Kretzschmar et al., 2015). Metabolites were measured in embryos and coleoptiles from a transgenic IR64 line expressing the KHO-*OsTPP7* gene and a near-isogenic line containing the KHO-*OsTPP7* locus introgressed into the IR64 background. These two lines were more capable of coleoptile elongation growth under anaerobiosis than the parental IR64 genotype and contained higher levels of trehalose and Suc than IR64. Although there were no significant differences in Tre6P content, the Tre6P:Suc ratio was decreased in both of the tolerant lines. It was proposed that enhanced mobilization of starch reserves in the endosperm of the tolerant genotypes maintains the supply of Suc to the growing embryos when the seeds germinate under anaerobic conditions (Kretzschmar et al., 2015).

Salinity is a serious and growing problem for agricultural production in many countries. Plants exposed to high salt levels must cope with the dual challenges of ion toxicity and osmotic stress. Several species have been observed to accumulate trehalose in response to salt stress, including wheat (El-Bashiti et al., 2005), rice (Shima et al., 2007), and *Medicago truncatula* (López et al., 2008). However, overall levels of trehalose in the salt-stressed plants were still relatively low, suggesting that trehalose made little direct contribution to osmoregulation, unless its distribution within the plants was concentrated in localized tissues or subcellular compartments. Salt tolerance in Arabidopsis is dependent on expression of AtTPPD, a redox-sensitive, chloroplastic isoform of TPP, suggesting that trehalose accumulation in chloroplasts could help to preserve their functionality under salt stress (Krasensky et al., 2014). In maize, salt stress induced changes in expression of several genes encoding enzymes and transporters involved in trehalose and Suc metabolism (Henry et al., 2015). The levels of many of the intermediates of sugar metabolism, glycolysis, and the tricarboxylic acid cycle were also affected, with a 2- to 4-fold increase in Tre6P levels in leaves, kernels, and cobs being observed at the silking stage. This suggests a rather general reconfiguration of gene expression and central metabolism in salt-stressed maize plants. It is not yet clear whether the increase in Tre6P at the earliest developmental stage (i.e. silking) represents a primary response to salt stress that drives other gene expression and metabolic changes or if the altered Tre6P levels and Tre6P:Suc ratios reflect the broader reconfiguration of metabolism in salt-stressed plants.

Apart from the extreme case of desiccation-tolerant resurrection plants, trehalose metabolism is also involved in responses to more moderate water deficits. Trehalose accumulation in response to water stress in wheat was associated with a decrease in trehalase activity (El-Bashiti et al., 2005; Kosmas et al., 2006). However, trehalose levels were still relatively low and unlikely to make much contribution to osmoregulation unless very localized. Overexpression of the yeast TPS (ScTPS1) in potato enhanced drought tolerance, but several known compatible solutes, including Pro, inositol, and raffinose, were increased in the ScTPS1-expressing line, so the improved drought tolerance may have been due to multiple factors (Kondrák et al., 2012).

Recently, expression of a rice TPP in developing maize ears was shown to improve yields under well-watered as well as drought conditions by preventing kernel abortion (Nuccio et al., 2015). The rice *OsTPP1* gene was expressed under the control of the rice *OsMADS6* gene promoter. This promoter drove expression in young maize florets before pollination and in developing kernels after pollination, the developmental stages when maize seed yield is most vulnerable to drought. Tre6P levels were decreased by about half in the ear spikelets under both drought stressed and nonstressed conditions, while Suc was increased under nonstressed conditions. In a commentary on the work of Nuccio et al. (2015), the simultaneous increase in Suc and decrease in Tre6P, presumably signaling low Suc, was considered to be puzzling (Guan and Koch, 2015). However, these reciprocal changes are exactly what would be predicted from the Suc-Tre6P nexus model, i.e. that overexpression of TPP would act to lower Tre6P levels, triggering a rise in Suc concentrations. It is unclear how decreasing Tre6P levels might bring about such a change in Suc concentrations. One possibility is that having low Tre6P in florets and developing kernels helps to maintain their ability to import Suc from the phloem. Another possibility is that

metabolism within the kernels is reconfigured to operate with higher Suc levels and a lower Tre6P:Suc ratio. Both scenarios would almost certainly involve other Suc signaling pathways and probably also hexose-signaling pathways, which would be affected by Tre6P-driven changes in Suc levels (Guan and Koch, 2015; Smeekens, 2015).

This landmark example of how to improve stress tolerance in a major crop species, with no yield penalty under nonstressed conditions, highlights both the potential of engineering trehalose metabolism for crop improvement and the likely need for spatio-temporally targeted interventions for a successful outcome (Nuccio et al., 2015). It also throws a spotlight on TPP. It is interesting to note that it is very often changes in expression of TPP that have been linked to improved stress tolerance in engineered lines and endogenous stress responses in wild-type plants. These include *OsTPP7* in the anaerobic germination tolerance of rice (Kretzschmar et al., 2015), *AtTPPA* and *AtTPPD* in cold and salt tolerance, respectively, in Arabidopsis (Iordachescu and Imai, 2008; Krasensky et al., 2014), *OsTPP1* and *OsTPP2* in cold stress responses in rice (Pramanik and Imai, 2005; Shima et al., 2007), and *VvTPPA* in chilling tolerance of grapevine (Fernandez et al., 2012).

The large family of functional *TPP* genes (*AtTPPA-AtTPPJ*) in Arabidopsis originates from serial genome duplication events and is unusual in showing a very high rate of paralog retention, a trait that is more common for genes encoding regulatory proteins than for enzyme genes (Vandesteene et al., 2012). Most of the *AtTPP* genes show developmental stage and tissue-specific expression patterns (Vandesteene et al., 2012), as does the *RAMOSA3* (*TPP*) gene in maize (Satoh-Nagasawa et al., 2006). We speculate that expression of different TPP isoforms could be a key factor in adapting the Suc-Tre6P nexus to the particular needs of different tissues, potentially modulating the response range and sensitivity of the Suc-Tre6P relationship. A systematic study of the kinetic (e.g. K_m for Tre6P, V_{max}) and regulatory properties of individual Arabidopsis TPP isoforms and further phenotypic analyses of Arabidopsis *tpp* mutants would help to test this hypothesis (see Outstanding Questions).

Tre6P AND REGULATION OF STOMATAL CONDUCTANCE

Within the leaves, trehalose metabolism plays a particularly important role in the regulation of stomatal conductance. In Arabidopsis, the *AtTPS1* gene is highly expressed is guard cells (Gómez et al., 2010), and these cells are one of the few examples where the AtTPS1 protein is abundant enough to have been detected in an untargeted proteomic analysis (Zhao et al., 2008). Expression of *AtTPPG* and *AtTREHALASE1* (*AtTRE1*; encoding the only known trehalase in Arabidopsis) is also particularly prominent in guard cells, and both genes are induced by ABA, which plays a key role in regulation of stomatal conductance (Vandesteene et al., 2012; Van Houtte et al., 2013). The Arabidopsis *tps1-12* mutant, which carries a weak allele of the *AtTPS1* gene, has a smaller stomatal aperture than wild-type plants (Gómez et al., 2010). Null *tppg*, *tre1-1*, and *tre1-2* mutants have stomata that are unresponsive to ABA, whereas AtTRE1 overexpressing plants are hypersensitive to exogenous ABA (Vandesteene et al., 2012; Van Houtte et al., 2013). These effects on the sensitivity of the stomata to ABA explain the paradoxical observation that the *tre1-1* and *tre1-2* null mutants are less drought tolerant than wild-type plants despite having higher levels of trehalose (Van Houtte et al., 2013), which is usually believed to confer resistance to drought and other abiotic stresses.

It is unknown at present how these lesions in trehalose metabolism affect stomatal sensitivity to ABA and whether Tre6P plays a direct role or only an indirect role as the precursor of trehalose. However, seed germination in the Arabidopsis *tppg*, *tre1-1*, and *tre1-2* mutants is also insensitive to inhibition by ABA, suggesting that there is a general defect in ABA signaling in these plants (Vandesteene et al., 2012; Van Houtte et al., 2013). Constitutive overexpression of *AtTPS1* also abolishes sensitivity of seed germination to ABA (Avonce et al., 2004), and interactions with trehalose metabolism have been noted for other ABA-mediated processes, such as potato tuber dormancy (Debast et al., 2011). Ser-Thr protein kinases from the SnRK2 family are major players in ABA signaling. These belong to the same SNF1/AMPK/SnRK superfamily of protein kinases as SnRK1, but from a different clade, and so far there have been no reports of direct effects of Tre6P on SnRK2 activity (Emanuelle et al., 2015). However, it was recently found that nitric oxide (NO) suppresses ABA signaling in guard cells by S-nitrosylation of SnRK2.6 (also known as OPEN STOMATA1; Wang et al., 2015). Furthermore, NO-mediated ABA signaling is impaired in *nia1 nia2* mutants, which lack NR activity, but restored by exogenous NO (Chen et al., 2016). The finding that Tre6P activates NR (Figueroa et al., 2016) suggests a possible role for Tre6P in ABA-mediated regulation of stomatal conductance, involving covalent modifications of NR and SnRK2.6, with NO as an intermediary signal (Fig. 6).

There are other ways that Tre6P might influence stomatal behavior. Guard cells are usually the only cells in the leaf epidermal layers that contain chloroplasts, but the photosynthetic capacity and physiological significance of guard cell chloroplasts have been a matter of debate for many years (Lawson et al., 2014). Guard cell-specific expression of chlorophyllase abolished the photosynthetic capacity of most guard cells but did not affect stomatal sensitivity to ABA or CO_2 stimuli (Azoulay-Shemer et al., 2015). However, guard cell turgor and energization were impaired, indicating that the photosynthetic capacity of guard cell chloroplasts is necessary for the guard cells to function properly.

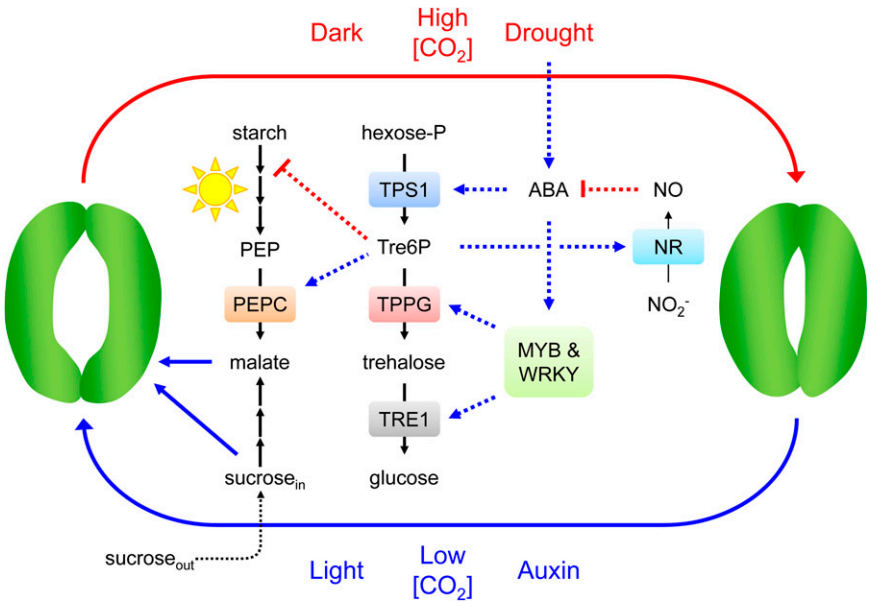

Figure 6. Trehalose metabolism in guard cells and regulation of stomatal conductance. *AtTPS1*, *AtTPPG*, and *AtTRE1* are particularly highly expressed in Arabidopsis guard cells, with their expression being up-regulated by ABA. The *AtTPPG* and *AtTRE1* gene promoters contain motifs that implicate MYB and WRKY family transcription factors in their regulation by ABA. The stomata in *tppg* and *tre1* null mutants are insensitive to ABA, rendering the plants less tolerant of water deficit (Vandesteene et al., 2012; Van Houtte et al., 2013). ABA sensitivity is compromised by NO, whose production is dependent on nitrate reductase (NR; Chen et al., 2016). Upon illumination, the starch in guard cells is rapidly degraded, providing substrates for synthesis of malate that helps drive stomatal opening (Horrer et al., 2016). In Arabidopsis mesophyll cells, high [Tre6P] leads to posttranslational activation of NR and phospho*enol*pyruvate carboxylase (PEPC) during the day and inhibition of transitory starch degradation at night (Martins et al., 2013; Figueroa et al., 2016), suggesting that the degradation of starch and synthesis of malate might also be regulated by Tre6P.

The pattern of starch turnover in guard cells is different to that in mesophyll cells; guard cell chloroplasts contain high levels of starch in the dark, and this is degraded in the light (Outlaw and Manchester, 1979; Rieger et al., 1992). Recent studies have shown that rapid degradation of starch is needed to drive stomatal opening in the light, in response to phototropin-mediated blue light signaling, and that degradation occurs via a distinct pathway involving α-AMYLASE3 and a redox-sensitive β-amylase, BAM1 (Valerio et al., 2011; Prasch et al., 2015; Horrer et al., 2016). It is not yet known whether Tre6P plays any role in regulation of starch degradation in guard cells. If it does, then its influence on guard cell starch metabolism might differ from the regulation of starch breakdown in mesophyll cells, given the specific functions and contrasting diurnal profiles of starch in the two cell types. Suc can be one of the major osmolytes involved in stomatal opening (Lawson et al., 2014; Daloso et al., 2016a, 2016b), so might Tre6P inhibit starch degradation in guard cells in the light if there is already sufficient Suc to drive stomatal opening? Conversely, might the distinct pathway of starch degradation in guard cells be insensitive to Tre6P, allowing starch to be degraded in the light even if Suc, and by implication Tre6P, levels are high? Without concrete evidence, we can only speculate on the answers to these questions (Fig. 6).

Rapid degradation of starch in guard cells provides substrates for glycolysis and production of PEP, which is carboxylated by PEPC to oxaloacetate, and then reduced to malate by malate dehydrogenase (Lawson et al., 2014). Malate is one of the major solutes that drives stomatal opening in many plants, acting as a counterion to K^+, and its synthesis via PEPC suggests a further potential regulatory role for Tre6P. At least three genes encoding PEPC are highly expressed in Arabidopsis guard cells: *PPC1* and *PPC2* encoding "plant-type" PEPC and *PPC4* encoding a "bacterial-type" PEPC. *PPC1* and *PPC2* also encode the major PEPC isoforms present in mesophyll cells, which were shown to be activated in response to induced increases in Tre6P (Figueroa et al., 2016). Therefore, it is possible that PEPC activity in guard cells is also subject to posttranslational regulation that is influenced by Tre6P (Fig. 6). Experiments to measure Tre6P and related metabolites (e.g. trehalose and Suc) in guard cells should have a high priority, to establish how Tre6P responds to the stimuli that affect stomatal opening and closing (e.g. light, $[CO_2]$, ABA, and auxin), and give clues to when and how Tre6P might be influencing stomatal function (see Outstanding Questions). Guard cell-specific manipulation of trehalose metabolism should also be a fruitful avenue of research.

CONCLUSION

The connection between trehalose and Suc metabolism is ancient, with phylogenetic analysis indicating that the two sugars have coexisted since the very beginning of the green plant lineage (Lunn, 2007). In the earliest plants, both sugars were probably used as osmolytes, carbon reserves, and stress protectants, functions that they still share today in extant primitive plants. For reasons that are not entirely clear, Suc eventually displaced trehalose during the evolution of vascular plants, becoming the main transport sugar, a major product of photosynthesis, and the starting point for C metabolism in sink tissues (Lunn, 2016). The dominance of Suc over trehalose in higher plants is nicely illustrated by stable-isotope ($^{13}CO_2$) labeling experiments, which showed that C flux into Suc in Arabidopsis leaves is up to 10,000 times higher than that into trehalose (Szecowka et al., 2013). As Suc came to dominate plant C metabolism, mechanisms to control the synthesis, transport, and consumption of Suc would have become ever more important. Even under favorable growth conditions, plants have to juggle their Suc metabolism in a three-way balancing act in order to (1) coordinate Suc synthesis in the leaves with photosynthetic CO_2 fixation, (2) control photoassimilate partitioning between Suc and starch to ensure the plant has enough C reserves to survive the night, and (3) match Suc supply from source tissues with Suc demand from growing sink organs (Stitt et al., 2010; Lunn, 2016). Environmental stresses impose yet further demands on the regulation of Suc metabolism. From the beginning of their coexistence in plants, the biosynthetic pathways for Suc6P/Suc and Tre6P/trehalose competed for substrates from a common pool of UDPG and hexose-phosphates in the cytosol. It is not too difficult to imagine how this competitive relationship between Suc and Tre6P might have evolved into one of mutual regulation, as conceived in the nexus model (Yadav et al., 2014).

The nexus model postulates that the principal function of Tre6P in plants is to signal and regulate Suc levels, providing a simple concept for understanding what Tre6P does in wild-type plants. The model also offers a framework for interpreting the pleiotropic phenotypes of mutants with altered Tre6P levels and for targeted engineering of Tre6P metabolism to improve crop plant productivity. In wild-type plants, high Suc levels will be accompanied by high levels of Tre6P and usually promote growth, i.e. high Tre6P will be associated with faster growth. However, overexpression of TPS in transgenic plants (e.g. *35S:otsA*) leads to elevated levels of Tre6P that can have a negative effect on growth because the artificially high Tre6P pushes Suc levels down, triggering low-Suc signaling pathways that restrict growth. In other words, high levels of Tre6P can be associated with negative as well as positive effects on growth, depending on the metabolic context and impact on Suc levels. Given the central importance of Suc in the life of higher plants, it is not surprising that tinkering with the Tre6P machinery that regulates Suc has such dramatic, and often confusing, effects on plant growth and development.

OUTSTANDING QUESTIONS

- How do fluctuations in Suc content give rise to changes in Tre6P levels?
- What are the kinetic and regulatory properties of the plant TPS and TPP enzymes?
- Do AtTPS1 and its orthologs in other species have noncatalytic functions?
- What are the functions of the class II TPS proteins (e.g. AtTPS5-AtTPS11) in plants?
- Where is Tre6P located within plant cells and how does it move between compartments?
- Is Tre6P a systemic signal of Suc status?
- How does Tre6P influence the post-translational regulation of PEP carboxylase and nitrate reductase?
- How does Tre6P regulate transitory starch breakdown in leaves at night?
- What is the relationship between Suc, Tre6P, and SnRK1 in sink organs, and how is this integrated with other signaling pathways to regulate growth?
- How does Tre6P influence stomatal function and abiotic stress tolerance?

A key feature of the nexus model is that the core relationship between Tre6P and Suc is flexible and can be adapted to the needs of individual tissues and circumstances by modulating the mechanisms that tie Tre6P and Suc together. For example, in source leaves, Tre6P exerts its influence on Suc levels mainly by affecting Suc synthesis (from photoassimilates during the day and from starch at night), whereas in sink organs, Tre6P regulates Suc consumption for growth. It is likely that the signaling outputs from Tre6P and Suc, beyond the core of the nexus, also differ between tissues and developmental stages, adding further nuances (and frustrating complexity) to the fundamental Suc-Tre6P relationship.

In conclusion, Suc plays a central role in the life of plants and its fate is inextricably intertwined with Tre6P. The Suc-Tre6P nexus model postulates that Tre6P is both a signal and negative feedback regulator of Suc levels and that these fundamental roles are adapted for the particular needs of individual tissues and circumstances. Tre6P emerged from obscurity only a few years ago, and we are still learning how to decipher the clues that will lead us to a proper understanding of its relationship with Suc. No doubt, many unexpected twists and turns lie ahead as we turn the pages of this fascinating tale of two sugars.

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LITERATURE CITED

Anselmino O, Gilg E (1913) Über das Vorkommen von Trehalose in Selaginella lepidophylla. Ber Deut Pharm Ges **23:** 326–330

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana.* Nature **408:** 796–815

Arrivault S, Guenther M, Ivakov A, Feil R, Vosloh D, van Dongen JT, Sulpice R, Stitt M (2009) Use of reverse-phase liquid chromatography, linked to tandem mass spectrometry, to profile the Calvin cycle and other metabolic intermediates in Arabidopsis rosettes at different carbon dioxide concentrations. Plant J **59:** 826–839

Arrivault S, Guenther M, Fry SC, Fuenfgeld MM, Veyel D, Mettler-Altmann T, Stitt M, Lunn JE (2015) Synthesis and use of stable isotope labelled internal standards for quantification of phosphorylated metabolites by LC-MS/MS. Anal Chem **87:** 6896–6904

Avonce N, Leyman B, Mascorro-Gallardo JO, Van Dijck P, Thevelein JM, Iturriaga G (2004) The Arabidopsis trehalose-6-P synthase AtTPS1 gene is a regulator of glucose, abscisic acid, and stress signaling. Plant Physiol **136:** 3649–3659

Avonce N, Mendoza-Vargas A, Morett E, Iturriaga G (2006) Insights on the evolution of trehalose biosynthesis. BMC Evol Biol **6:** 109–123

Avonce N, Wuyts J, Verschooten K, Vandesteene L, Van Dijck P (2010) The *Cytophaga hutchinsonii* ChTPSP: first characterized bifunctional TPS-TPP protein as putative ancestor of all eukaryotic trehalose biosynthesis proteins. Mol Biol Evol **27:** 359–369

Azoulay-Shemer T, Palomares A, Bagheri A, Israelsson-Nordstrom M, Engineer CB, Bargmann BOR, Stephan AB, Schroeder JI (2015) Guard cell photosynthesis is critical for stomatal turgor production, yet does not directly mediate CO_2- and ABA-induced stomatal closing. Plant J **83:** 567–581

Bachmann M, McMichael RW Jr, Huber JL, Kaiser WM, Huber SC (1995) Partial purification and characterization of a calcium-dependent protein kinase and an inhibitor protein required for inactivation of spinach leaf nitrate reductase. Plant Physiol **108:** 1083–1091

Bachmann M, Shiraishi N, Campbell WH, Yoo BC, Harmon AC, Huber SC (1996) Identification of Ser-543 as the major regulatory phosphorylation site in spinach leaf nitrate reductase. Plant Cell **8:** 505–517

Baena-González E, Rolland F, Thevelein JM, Sheen J (2007) A central integrator of transcription networks in plant stress and energy signalling. Nature **448:** 938–942

Bailey-Serres J, Fukao T, Gibbs DJ, Holdsworth MJ, Lee SC, Licausi F, Perata P, Voesenek LA, van Dongen JT (2012) Making sense of low oxygen sensing. Trends Plant Sci **17:** 129–138

Barratt DH, Derbyshire P, Findlay K, Pike M, Wellner N, Lunn J, Feil R, Simpson C, Maule AJ, Smith AM (2009) Normal growth of Arabidopsis requires cytosolic invertase but not sucrose synthase. Proc Natl Acad Sci USA **106:** 13124–13129

Bechtold U, Penfold CA, Jenkins DJ, Legaie R, Moore JD, Lawson T, Matthews JSA, Vialet-Chabrand SRM, Baxter L, Subramaniam S, et al (2016) Time-series transcriptomics reveals that AGAMOUS-LIKE22 affects primary metabolism and developmental processes in drought-stressed Arabidopsis. Plant Cell **28:** 345–366

Benaroudj N, Lee DH, Goldberg AL (2001) Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. J Biol Chem **276:** 24261–24267

Bihmidine S, Hunter CT, Johns CE, Koch KE, Braun DM (2013) Regulation of assimilate import into sink organs: update on molecular drivers of sink strength. Fron. Plant Sci **4:** e177

Blázquez MA, Santos E, Flores C-L, Martínez-Zapater JM, Salinas J, Gancedo C (1998) Isolation and molecular characterization of the Arabidopsis TPS1 gene, encoding trehalose-6-phosphate synthase. Plant J **13:** 685–689

Budde RJ, Randall DD (1990) Pea leaf mitochondrial pyruvate dehydrogenase complex is inactivated *in vivo* in a light-dependent manner. Proc Natl Acad Sci USA **87:** 673–676

Cabib E, Leloir LF (1958) The biosynthesis of trehalose phosphate. J Biol Chem **231:** 259–275

Carroll L (1898). The Hunting of the Snark: An Agony in Eight Fits. The Macmillan Company, London.

Carillo P, Feil R, Gibon Y, Satoh-Nagasawa N, Jackson D, Bläsing OE, Stitt M, Lunn JE (2013) A fluorometric assay for trehalose in the picomole range. Plant Methods **9:** 21

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA, Penfield S (2014) Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year. Proc Natl Acad Sci USA **111:** 18787–18792

Chen Y, Hoehenwarter W (2015) Changes in the phosphoproteome and metabolome link early signaling events to rearrangement of photosynthesis and central metabolism in salinity and oxidative stress response in Arabidopsis. Plant Physiol **169:** 3021–3033

Chen ZH, Wang Y, Wang JW, Babla M, Zhao C, García-Mata C, Sani E, Differ C, Mak M, Hills A, Amtmann A, Blatt MR (2016) Nitrate reductase mutation alters potassium nutrition as well as nitric oxide-mediated control of guard cell ion channels in Arabidopsis. New Phytol **209:** 1456–1469

Cookson SJ, Yadav UP, Klie S, Morcuende R, Usadel B, Lunn JE, Stitt M (2016) Temporal kinetics of the transcriptional response to carbon depletion and sucrose readdition in Arabidopsis seedlings. Plant Cell Environ **39:** 768–786

Cortina C, Culianez-Macia FA (2005) Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. Plant Sci **169:** 75–82

Couée I, Sulmon C, Gouesbet G, El Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J Exp Bot **57:** 449–459

Dai ZW, Léon C, Feil R, Lunn JE, Delrot S, Gomès E (2013) Metabolic profiling reveals coordinated switches in primary carbohydrate metabolism in grape berry (*Vitis vinifera* L.), a non-climacteric fleshy fruit. J Exp Bot **64:** 1345–1355

Daloso DM, Williams TCR, Antunes WC, Pinheiro DP, Müller C, Loureiro ME, Fernie AR (2016a) Guard cell-specific upregulation of *sucrose synthase 3* reveals that the role of sucrose in stomatal function is primarily energetic. New Phytol **209:** 1470–1483

Daloso DM, Dos Anjos L, Fernie AR (2016b) Roles of sucrose in guard cell regulation. New Phytol **211:** 809–818

Debast S, Nunes-Nesi A, Hajirezaei MR, Hofmann J, Sonnewald U, Fernie AR, Börnke F (2011) Altering trehalose-6-phosphate content in transgenic potato tubers affects tuber growth and alters responsiveness to hormones during sprouting. Plant Physiol **156:** 1754–1771

Delatte TL, Sedijani P, Kondou Y, Matsui M, de Jong GJ, Somsen GW, Wiese-Klinkenberg A, Primavesi LF, Paul MJ, Schluepmann H (2011) Growth arrest by trehalose-6-phosphate: an astonishing case of primary metabolite control over growth by way of the SnRK1 signaling pathway. Plant Physiol **157:** 160–174

Delorge I, Figueroa CM, Feil R, Lunn JE, Van Dijck P (2015) Trehalose-6-phosphate synthase 1 is not the only active TPS in *Arabidopsis thaliana.* Biochem J **466:** 283–290

Djonović S, Urbach JM, Drenkard E, Bush J, Feinbaum R, Ausubel JL, Traficante D, Risech M, Kocks C, Fischbach MA, Priebe GP, Ausubel FM (2013) Trehalose biosynthesis promotes *Pseudomonas aeruginosa* pathogenicity in plants. PLoS Pathog **9:** e1003217

Dobrenel T, Marchive C, Azzopardi M, Clément G, Moreau M, Sormani R, Robaglia C, Meyer C (2013) Sugar metabolism and the plant target of rapamycin kinase: a sweet operaTOR? Front Plant Sci **4:** 93

Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016) TOR signaling and nutrient sensing. Annu Rev Plant Biol **67:** 261–285

Drennan PM, Smith MT, Goldsworth D, Van Staden J (1993) The occurrence of trehalose in the leaves of the desiccation-tolerant angiosperm *Myrothamnus flabellifolius* Welw. J Plant Physiol **142:** 493–496

Eastmond PJ, van Dijken AJH, Spielman M, Kerr A, Tissier AF, Dickinson HG, Jones JDG, Smeekens SC, Graham IA (2002) Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. Plant J **29:** 225–235

Edner C, Li J, Albrecht T, Mahlow S, Hejazi M, Hussain H, Kaplan F, Guy C, Smith SM, Steup M, Ritte G (2007) Glucan, water dikinase activity stimulates breakdown of starch granules by plastidial β-amylases. Plant Physiol **145:** 17–28

El-Bashiti T, Hamamci H, Oktem HA, Yucel M (2005) Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions. Plant Sci **169:** 47–54

Elbein AD (1974) The metabolism of α,α-trehalose. Adv Carbohydr Chem Biochem **30:** 227–257

Emanuelle S, Hossain MI, Moller IE, Pedersen HL, van de Meene AM, Doblin MS, Koay A, Oakhill JS, Scott JW, Willats WG, et al (2015) SnRK1 from *Arabidopsis thaliana* is an atypical AMPK. Plant J **82:** 183–192

Farrant JM, Cooper K, Hilgart A, Abdalla KO, Bentley J, Thomson JA, Dace HJ, Peton N, Mundree SG, Rafudeen MS (2015) A molecular physiological review of vegetative desiccation tolerance in the resurrection plant *Xerophyta viscosa* (Baker). Planta **242:** 407–426

Fernandez O, Vandesteene L, Feil R, Baillieul F, Lunn JE, Clément C (2012) Trehalose metabolism is activated upon chilling in grapevine and might participate in *Burkholderia phytofirmans* induced chilling tolerance. Planta **236:** 355–369

Fernandez O, Béthencourt L, Quero A, Sangwan RS, Clément C (2010) Trehalose and plant stress responses: friend or foe? Trends Plant Sci **15:** 409–417

Foster AJ, Jenkinson JM, Talbot NJ (2003) Trehalose synthesis and metabolism are required at different stages of plant infection by *Magnaporthe grisea*. EMBO J **22:** 225–235

Freeman BC, Chen C, Beattie GA (2010) Identification of the trehalose biosynthetic loci of *Pseudomonas syringae* and their contribution to fitness in the phyllosphere. Environ Microbiol **12:** 1486–1497

Figueroa CM, Feil R, Ishihara H, Watanabe M, Kölling K, Krause U, Höhne M, Encke B, Plaxton WC, Zeeman SC, et al (2016) Trehalose 6-phosphate coordinates organic and amino acid metabolism with carbon availability. Plant J **85:** 410–423

Fu Y, Lim S, Urano D, Tunc-Ozdemir M, Phan NG, Elston TC, Jones AM (2014) Reciprocal encoding of signal intensity and duration in a glucose-sensing circuit. Cell **156:** 1084–1095

Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Kochian LV, Wu RJ (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci USA **99:** 15898–15903

Gauthier PP, Bligny R, Gout E, Mahé A, Nogués S, Hodges M, Tcherkez GG (2010) *In folio* isotopic tracing demonstrates that nitrogen assimilation into glutamate is mostly independent from current CO_2 assimilation in illuminated leaves of *Brassica napus*. New Phytol **185:** 988–999

Geelen D, Royackers K, Vanstraelen M, De Bus M, Inzé D, Van Dijck P, Thevelein JM, Leyman B (2007) Trehalose-6-P synthase AtTPS1 high molecular weight complexes in yeast and Arabidopsis. Plant Sci **173:** 426–437

Gemel J, Randall DD (1992) Light regulation of leaf mitochondrial pyruvate dehydrogenase complex : role of photorespiratory carbon metabolism. Plant Physiol **100:** 908–914

Goddijn OJM, van Dun K (1999) Trehalose metabolism in plants. Trends Plant Sci **4:** 315–319

Goddijn OJM, Verwoerd TC, Voogd E, Krutwagen RWHH, de Graaf PTHM, Poels J, van Dun K, Ponstein AS, Damm B, Pen J (1997) Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol **113:** 181–190

Gómez LD, Baud S, Gilday A, Li Y, Graham IA (2006) Delayed embryo development in the ARABIDOPSIS TREHALOSE-6-PHOSPHATE SYNTHASE 1 mutant is associated with altered cell wall structure, decreased cell division and starch accumulation. Plant J **46:** 69–84

Gómez LD, Gilday A, Feil R, Lunn JE, Graham IA (2010) AtTPS1-mediated trehalose 6-phosphate synthesis is essential for embryogenic and vegetative growth and responsiveness to ABA in germinating seeds and stomatal guard cells. Plant J **64:** 1–13

Graf A, Schlereth A, Stitt M, Smith AM (2010) Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. Proc Natl Acad Sci USA **107:** 9458–9463

Guan JC, Koch KE (2015) A time and a place for sugar in your ears. Nat Biotechnol **33:** 827–828

Gussin AES (1972) Does trehalose occur in Angiospermae? Phytochemistry **11:** 1827–1828

Hädrich N, Hendriks JHM, Kötting O, Arrivault S, Feil R, Zeeman SC, Gibon Y, Schulze WX, Stitt M, Lunn JE (2012) Mutagenesis of cysteine 81 prevents dimerization of the APS1 subunit of ADP-glucose pyrophosphorylase and alters diurnal starch turnover in *Arabidopsis thaliana* leaves. Plant J **70:** 231–242

Hanson J, Hanssen M, Wiese A, Hendriks MM, Smeekens S (2008) The sucrose regulated transcription factor bZIP11 affects amino acid metabolism by regulating the expression of ASPARAGINE SYNTHETASE1 and PROLINE DEHYDROGENASE2. Plant J **53:** 935–949

Harthill JE, Meek SEM, Morrice N, Peggie MW, Borch J, Wong BHC, Mackintosh C (2006) Phosphorylation and 14-3-3 binding of Arabidopsis trehalose-phosphate synthase 5 in response to 2-deoxyglucose. Plant J **47:** 211–223

Hartwell J, Gill A, Nimmo GA, Wilkins MB, Jenkins GI, Nimmo HG (1999) Phosphoenolpyruvate carboxylase kinase is a novel protein kinase regulated at the level of expression. Plant J **20:** 333–342

Haydon MJ, Mielczarek O, Robertson FC, Hubbard KE, Webb AAR (2013) Photosynthetic entrainment of the *Arabidopsis thaliana* circadian clock. Nature **502:** 689–692

Hejazi M, Fettke J, Kötting O, Zeeman SC, Steup M (2010) The Laforin-like dual-specificity phosphatase SEX4 from Arabidopsis hydrolyzes both C6- and C3-phosphate esters introduced by starch-related dikinases and thereby affects phase transition of α-glucans. Plant Physiol **152:** 711–722

Hendriks JHM, Kolbe A, Gibon Y, Stitt M, Geigenberger P (2003) ADP-glucose pyrophosphorylase is activated by posttranslational redox-modification in response to light and to sugars in leaves of Arabidopsis and other plant species. Plant Physiol **133:** 838–849

Henry C, Bledsoe SW, Griffiths CA, Kollman A, Paul MJ, Sakr S, Lagrimini LM (2015) Differential role for trehalose metabolism in salt-stressed maize. Plant Physiol **169:** 1072–1089

Henry C, Bledsoe SW, Siekman A, Kollman A, Waters BM, Feil R, Stitt M, Lagrimini LM (2014) The trehalose pathway in maize: conservation and gene regulation in response to the diurnal cycle and extended darkness. J Exp Bot **65:** 5959–5973

Horrer D, Flütsch S, Pazmino D, Matthews JS, Thalmann M, Nigro A, Leonhardt N, Lawson T, Santelia D (2016) Blue light induces a distinct starch degradation pathway in guard cells for stomatal opening. Curr Biol **26:** 362–370

Huber SC, Huber JL (1996) Role and regulation of sucrose-phosphate synthase in higher plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 431–444

Iordachescu M, Imai R (2008) Trehalose biosynthesis in response to abiotic stresses. J Integr Plant Biol **50:** 1223–1229

Ishihara H, Obata T, Sulpice R, Fernie AR, Stitt M (2015) Quantifying protein synthesis and degradation in Arabidopsis by dynamic $^{13}CO_2$ labeling and analysis of enrichment in individual amino acids in their free pools and in protein. Plant Physiol **168:** 74–93

Iturriaga G, Gaff DF, Zentella R (2000) New desiccation-tolerant plants, including a grass, in the central high-lands of Mexico, accumulate trehalose. Aust J Bot **48:** 153–158

Kaiser WM, Huber SC (2001) Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. J Exp Bot **52:** 1981–1989

Kandler O, Hopf H (1980) Occurrence, metabolism and function of oligosaccharides. *In* J Preiss, ed, The Biochemistry of Plants – A Comprehensive Treatise. Carbohydrates – Structure and Function, Vol 3. Academic Press, New York, pp 221–270

Kolbe A, Tiessen A, Schluepmann H, Paul M, Ulrich S, Geigenberger P (2005) Trehalose 6-phosphate regulates starch synthesis via posttranslational redox activation of ADP-glucose pyrophosphorylase. Proc Natl Acad Sci USA **102:** 11118–11123

Kondrák M, Marincs F, Antal F, Juhász Z, Bánfalvi Z (2012) Effects of yeast trehalose-6-phosphate synthase 1 on gene expression and carbohydrate contents of potato leaves under drought stress conditions. BMC Plant Biol **12:** 74

Kosmas SA, Argyrokastritis A, Loukas MG, Eliopoulos E, Tsakas S, Kaltsikes PJ (2006) Isolation and characterization of drought-related trehalose 6-phosphate-synthase gene from cultivated cotton (*Gossypium hirsutum* L.). Planta **223:** 329–339

Kötting O, Pusch K, Tiessen A, Geigenberger P, Steup M, Ritte G (2005) Identification of a novel enzyme required for starch metabolism in Arabidopsis leaves. The phosphoglucan, water dikinase. Plant Physiol **137:** 242–252

Kötting O, Santelia D, Edner C, Eicke S, Marthaler T, Gentry MS, Comparot-Moss S, Chen J, Smith AM, Steup M, Ritte G, Zeeman SC (2009) STARCH-EXCESS4 is a laforin-like Phosphoglucan phosphatase required for starch degradation in *Arabidopsis thaliana*. Plant Cell **21:** 334–346

Krasensky J, Broyart C, Rabanal FA, Jonak C (2014) The redox-sensitive chloroplast trehalose-6-phosphate phosphatase AtTPPD regulates salt stress tolerance. Antioxid Redox Signal **21:** 1289–1304

Kretzschmar T, Pelayo MAF, Trijatmiko KR, Gabunada LFM, Alam R, Jimenez R, Mendioro MS, Slamet-Loedin IH, Sreenivasulu N, Bailey-Serres J, et al (2015) A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nat Plants **1:** 15124

Lawson T, Simkin AJ, Kelly G, Granot D (2014) Mesophyll photosynthesis and guard cell metabolism impacts on stomatal behaviour. New Phytol **203:** 1064–1081

Le BH, Cheng C, Bui AQ, Wagmaister JA, Henry KF, Pelletier J, Kwong L, Belmonte M, Kirkbride R, Horvath S, et al (2010) Global analysis of gene activity during Arabidopsis seed development and identification of seed-specific transcription factors. Proc Natl Acad Sci USA **107:** 8063–8070

Lee SB, Kwon HB, Kwon SJ, Park SC, Jeong MJ, Han SE, Byun MO, Henry D (2003) Accumulation of trehalose within transgenic chloroplasts confers drought tolerance. Mol Breed **11:** 1–13

Leyman B, Van Dijck P, Thevelein JM (2001) An unexpected plethora of trehalose biosynthesis genes in *Arabidopsis thaliana.* Trends Plant Sci **6:** 510–513

Li H-W, Zang B-S, Deng X-W, Wang X-P (2011) Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. Planta **234:** 1007–1018

Li J, Almagro G, Muñoz FJ, Baroja-Fernández E, Bahaji A, Montero M, Hidalgo M, Sánchez-López AM, Ezquer I, Sesma MT, Pozueta-Romero J (2012) Post-translational redox modification of ADP-glucose pyrophosphorylase in response to light is not a major determinant of fine regulation of transitory starch accumulation in Arabidopsis leaves. Plant Cell Physiol **53:** 433–444

Lilley JLS, Gee CW, Sairanen I, Ljung K, Nemhauser JL (2012) An endogenous carbon-sensing pathway triggers increased auxin flux and hypocotyl elongation. Plant Physiol **160:** 2261–2270

López M, Herrera-Cervera JA, Iribarne C, Tejera NA, Lluch C (2008) Growth and nitrogen fixation in *Lotus japonicus* and *Medicago truncatula* under NaCl stress: nodule carbon metabolism. J Plant Physiol **165:** 641–650

Lunn JE (2016) Sucrose metabolism *In* AM Hetherington, ed, Encyclopedia of Life Science (ELS). John Wiley & Sons, Chichester, UK.

Lunn JE (2007) Gene families and evolution of trehalose metabolism in plants. Funct Plant Biol **34:** 550–563

Lunn JE, Delorge I, Figueroa CM, Van Dijck P, Stitt M (2014) Trehalose metabolism in plants. Plant J **79:** 544–567

Lunn JE, Feil R, Hendriks JHM, Gibon Y, Morcuende R, Osuna D, Scheible W-R, Carillo P, Hajirezaei M-R, Stitt M (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in *Arabidopsis thaliana.* Biochem J **397:** 139–148

Luo Y, Li WM, Wang W (2008) Trehalose: Protector of antioxidant enzymes or reactive oxygen species scavenger under heat stress? Environ Exp Bot **63:** 378–384

Ma J, Hanssen M, Lundgren K, Hernández L, Delatte T, Ehlert A, Liu CM, Schluepmann H, Dröge-Laser W, Moritz T, Smeekens S, Hanson J (2011) The sucrose-regulated Arabidopsis transcription factor bZIP11 reprograms metabolism and regulates trehalose metabolism. New Phytol **191:** 733–745

Martínez-Barajas E, Delatte T, Schluepmann H, de Jong GJ, Somsen GW, Nunes C, Primavesi LF, Coello P, Mitchell RA, Paul MJ (2013) Wheat grain development is characterized by remarkable trehalose 6-phosphate accumulation pregrain filling: tissue distribution and relationship to SNF1-related protein kinase1 activity. Plant Physiol **156:** 373–381

Martins MCM, Hejazi M, Fettke J, Steup M, Feil R, Krause U, Arrivault S, Vosloh D, Figueroa CM, Ivakov A, et al (2013) Feedback inhibition of starch degradation in Arabidopsis leaves mediated by trehalose 6-phosphate. Plant Physiol **163:** 1142–1163

Ming R, Hou S, Feng Y, Yu Q, Dionne-Laporte A, Saw JH, Senin P, Wang W, Ly BV, Lewis KL, et al (2008) The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). Nature **452:** 991–996

Miranda JA, Avonce N, Suárez R, Thevelein JM, Van Dijck P, Iturriaga G (2007) A bifunctional TPS-TPP enzyme from yeast confers tolerance to multiple and extreme abiotic-stress conditions in transgenic Arabidopsis. Planta **226:** 1411–1421

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science **300:** 332–336

Mugford ST, Fernandez O, Brinton J, Flis A, Krohn N, Encke B, Feil R, Sulpice R, Lunn JE, Stitt M, Smith AM (2014) Regulatory properties of ADP glucose pyrophosphorylase are required for adjustment of leaf starch synthesis in different photoperiods. Plant Physiol **166:** 1733–1747

Nelson DL, Cox MM (2013) Lehninger Principles of Biochemistry, Ed 6. W. H. Freeman & Co., New York

Niittylä T, Comparot-Moss S, Lue WL, Messerli G, Trevisan M, Seymour MD, Gatehouse JA, Villadsen D, Smith SM, Chen J, Zeeman SC, Smith AM (2006) Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. J Biol Chem **281:** 11815–11818

Nuccio ML, Wu J, Mowers R, Zhou H-P, Meghji M, Primavesi LF, Paul MJ, Chen X, Gao Y, Haque E, Basu SS, Lagrimini LM (2015) Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. Nat Biotechnol **33:** 862–869

Nunes C, O'Hara LE, Primavesi LF, Delatte TL, Schluepmann H, Somsen GW, Silva AB, Fevereiro PS, Wingler A, Paul MJ (2013a) The trehalose 6-phosphate/SnRK1 signaling pathway primes growth recovery following relief of sink limitation. Plant Physiol **162:** 1720–1732

Nunes C, Primavesi LF, Patel MK, Martinez-Barajas E, Powers SJ, Sagar R, Fevereiro PS, Davis BG, Paul MJ (2013b) Inhibition of SnRK1 by metabolites: tissue-dependent effects and cooperative inhibition by glucose 1-phosphate in combination with trehalose 6-phosphate. Plant Physiol Biochem **63:** 89–98

Nunes C, Schluepmann H, Delatte TL, Wingler A, Silva AB, Fevereio PS, Jansen M, Fiorani F, Wiese-Klinkenberg A, Paul M (2013c) Regulation of growth by the trehalose pathway: relationship to temperature and sucrose. Plant Signal Behav **8:** 12

O'Hara LE, Paul MJ, Wingler A (2013) How do sugars regulate plant growth and development? New insight into the role of trehalose-6-phosphate. Mol Plant **6:** 261–274

O'Leary B, Park J, Plaxton WC (2011) The remarkable diversity of plant PEPC (phosphoenolpyruvate carboxylase): recent insights into the physiological functions and post-translational controls of non-photosynthetic PEPCs. Biochem J **436:** 15–34

Outlaw WH, Manchester J (1979) Guard cell starch concentration quantitatively related to stomatal aperture. Plant Physiol **64:** 79–82

Paul MJ, Jhurreea D, Zhang Y, Primavesi LF, Delatte T, Schluepmann H, Wingler A (2010) Upregulation of biosynthetic processes associated with growth by trehalose 6-phosphate. Plant Signal Behav **5:** 386–392

Piazza A, Zimaro T, Garavaglia BS, Ficarra FA, Thomas L, Marondedze C, Feil R, Lunn JE, Gehring C, Ottado J, Gottig N (2015) The dual nature of trehalose in citrus canker disease: a virulence factor for *Xanthomonas citri* subsp. *citri* and a trigger for plant defence responses. J Exp Bot **66:** 2795–2811

Pilon-Smits EAH, Terry N, Sears T, Kim H, Zayed A, Hwang S, Van Dun K, Voogd E, Verwoerd TC, Krutwagen RWHH, Goddijn OJM (1998) Trehalose producing transgenic tobacco plants show improved growth performance under drought stress. J Plant Physiol **152:** 525–532

Pramanik MHR, Imai R (2005) Functional identification of a trehalose 6-phosphate phosphatase gene that is involved in transient induction of trehalose biosynthesis during chilling stress in rice. Plant Mol Biol **58:** 751–762

Prasch CM, Ott KV, Bauer H, Ache P, Hedrich R, Sonnewald U (2015) β-Amylase1 mutant Arabidopsis plants show improved drought tolerance due to reduced starch breakdown in guard cells. J Exp Bot **66:** 6059–6067

Radchuk R, Emery RJN, Weier D, Vigeolas H, Geigenberger P, Lunn JE, Feil R, Weschke W, Weber H (2010) Sucrose non-fermenting kinase 1 (SnRK1) coordinates metabolic and hormonal signals during pea cotyledon growth and differentiation. Plant J **61:** 324–338

Ragel P, Streb S, Feil R, Sahrawy M, Annunziata MG, Lunn JE, Zeeman S, Mérida Á (2013) Loss of starch granule initiation has a deleterious effect on the growth of arabidopsis plants due to an accumulation of ADP-glucose. Plant Physiol **163:** 75–85

Rahmani F, Hummel M, Schuurmans J, Wiese-Klinkenberg A, Smeekens S, Hanson J (2009) Sucrose control of translation mediated by an upstream open reading frame-encoded peptide. Plant Physiol **150:** 1356–1367

Ramon M, De Smet I, Vandesteene L, Naudts M, Leyman B, Van Dijck P, Rolland F, Beeckman T, Thevelein JM (2009) Extensive expression regulation and lack of heterologous enzymatic activity of the Class II trehalose metabolism proteins from *Arabidopsis thaliana.* Plant Cell Environ **32:** 1015–1032

Reignault PH, Cogan A, Muchembled J, Lounes-Hadj Sahraoui A, Durand R, Sancholle M (2001) Trehalose induces resistance to powdery mildew in wheat. New Phytol **149:** 519–529

Renard-Merlier D, Randoux B, Nowak E, Farcy F, Durand R, Reignault P (2007) Iodus 40, salicylic acid, heptanoyl salicylic acid and trehalose exhibit different efficacies and defence targets during a wheat/powdery mildew interaction. Phytochemistry **68:** 1156–1164

Rieger A, Lutz A, Hampp R (1992) Compartmentation of soluble carbohydrates, of starch and of malate in motor organs (pulvini) and other parts of *Phaseolus coccineus* L. leaves. Planta **187:** 95–102

Ritte G, Lloyd JR, Eckermann N, Rottmann A, Kossmann J, Steup M (2002) The starch-related R1 protein is an α-glucan, water dikinase. Proc Natl Acad Sci USA **99:** 7166–7171

Ritte G, Heydenreich M, Mahlow S, Haebel S, Kötting O, Steup M (2006) Phosphorylation of C6- and C3-positions of glucosyl residues in starch is catalysed by distinct dikinases. FEBS Lett **580:** 4872–4876

Rodríguez-Salazar J, Suárez R, Caballero-Mellado J, Iturriaga G (2009) Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants. FEMS Microbiol Lett **296:** 52–59

Romero C, Bellés JM, Vayá JL, Serrano R, Culiáñez-Macià FA (1997) Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. Planta **201:** 293–297

Romero C, Cruz Cutanda M, Cortina C, Primo J, Culiáñez-Macià F (2002) Plant environmental stress response by trehalose biosynthesis. Curr Topics Plant Biol **3:** 73–88

Sairanen I, Novák O, Pěnčík A, Ikeda Y, Jones B, Sandberg G, Ljung K (2012) Soluble carbohydrates regulate auxin biosynthesis via PIF proteins in Arabidopsis. Plant Cell **24:** 4907–2916

Santelia D, Kötting O, Seung D, Schubert M, Thalmann M, Bischof S, Meekins DA, Lutz A, Patron N, Gentry MS, Allain FH, Zeeman SC (2011) The phosphoglucan phosphatase like sex Four2 dephosphorylates starch at the C3-position in Arabidopsis. Plant Cell **23:** 4096–4111

Sastre Toraño J, Delatte TL, Schluepmann H, Smeekens SC, de Jong GJ, Somsen GW (2012) Determination of trehalose-6-phosphate in *Arabidopsis thaliana* seedlings by hydrophilic-interaction liquid chromatography-mass spectrometry. Anal Bioanal Chem **403:** 1353–1360

Satoh-Nagasawa N, Nagasawa N, Malcomber S, Sakai H, Jackson D (2006) A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature **441:** 227–230

Schluepmann H, Pellny T, van Dijken A, Smeekens S, Paul M (2003) Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in *Arabidopsis thaliana.* Proc Natl Acad Sci USA **100:** 6849–6854

Schmid M, Davison TS, Henz SR, Pape UJ, Demar M, Vingron M, Schölkopf B, Weigel D, Lohmann JU (2005) A gene expression map of *Arabidopsis thaliana* development. Nat Genet **37:** 501–506

Scialdone A, Mugford ST, Feike D, Skeffington A, Borrill P, Graf A, Smith AM, Howard M (2013) Arabidopsis plants perform arithmetic division to prevent starvation at night. eLife **2:** e00669

Shane MW, Feil R, Lunn JE, Plaxton WC (2016) Light-dependent activation of phosphoenolpyruvate carboxylase by reversible phosphorylation in cluster roots of white lupin plants: diurnal control in response to photosynthate supply. Ann Bot (Lond) **pii:** mcw040

Shima S, Matsui H, Tahara S, Imai R (2007) Biochemical characterization of rice trehalose-6-phosphate phosphatases supports distinctive functions of these plant enzymes. FEBS J **274:** 1192–1201

Singh V, Louis J, Ayre BG, Reese JC, Pegadaraju V, Shah J (2011) TREHALOSE PHOSPHATE SYNTHASE11-dependent trehalose metabolism promotes *Arabidopsis thaliana* defense against the phloem-feeding insect *Myzus persicae.* Plant J **67:** 94–104

Smeekens S (2015) From leaf to kernel: trehalose-6-phosphate signaling moves carbon in the field. Plant Physiol **169:** 912–913

Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. Curr Opin Plant Biol **13:** 274–279

Stiller I, Dulai S, Kondrák M, Tarnai R, Szabó L, Toldi O, Bánfalvi Z (2008) Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of *Saccharomyces cerevisiae.* Planta **227:** 299–308

Stitt M, Lunn J, Usadel B (2010) Arabidopsis and primary photosynthetic metabolism - more than the icing on the cake. Plant J **61:** 1067–1091

Stitt M, Zeeman SC (2012) Starch turnover: pathways, regulation and role in growth. Curr Opin Plant Biol **15:** 282–292

Su W, Huber SC, Crawford NM (1996) Identification *in vitro* of a post-translational regulatory site in the hinge 1 region of *Arabidopsis* nitrate reductase. Plant Cell **8:** 519–527

Sugden C, Donaghy PG, Halford NG, Hardie DG (1999) Two SNF1-related protein kinases from spinach leaf phosphorylate and inactivate 3-hydroxy-3-methylglutaryl coenzyme A reductase, nitrate reductase, and sucrose phosphate synthase in vitro. Plant Physiol **120:** 257–274

Sulpice R, Flis A, Ivakov AA, Apelt F, Krohn N, Encke B, Abel C, Feil R, Lunn JE, Stitt M (2014) Arabidopsis coordinates the diurnal regulation of carbon allocation and growth across a wide range of photoperiods. Mol Plant **7:** 137–155

Suárez R, Wong A, Ramírez M, Barraza A, Orozco MdelC, Cevallos MA, Lara M, Hernández G, Iturriaga G (2008) Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate synthase in rhizobia. Mol Plant Microbe Interact **21:** 958–966

Suzuki N, Bajad S, Shuman J, Shulaev V, Mittler R (2008) The transcriptional co-activator MBF1c is a key regulator of thermotolerance in *Arabidopsis thaliana.* J Biol Chem **283:** 9269–9275

Szecowka M, Heise R, Tohge T, Nunes-Nesi A, Vosloh D, Huege J, Feil R, Lunn J, Nikoloski Z, Stitt M, Fernie AR, Arrivault S (2013) Metabolic fluxes in an illuminated Arabidopsis rosette. Plant Cell **25:** 694–714

Tapia H, Young L, Fox D, Bertozzi CR, Koshland D (2015) Increasing intracellular trehalose is sufficient to confer desiccation tolerance to *Saccharomyces cerevisiae.* Proc Natl Acad Sci USA **112:** 6122–6127

Tayeh C, Randoux B, Vincent D, Bourdon N, Reignault P (2014) Exogenous trehalose induces defenses in wheat before and during a biotic stress caused by powdery mildew. Phytopathology **104:** 293–305

Tcherkez G, Mahé A, Guérard F, Boex-Fontvieille ER, Gout E, Lamothe M, Barbour MM, Bligny R (2012) Short-term effects of CO_2 and O_2 on citrate metabolism in illuminated leaves. Plant Cell Environ **35:** 2208–2220

Thiel J, Rolletschek H, Friedel S, Lunn JE, Nguyen TH, Feil R, Tschiersch H, Müller M, Borisjuk L (2011) Seed-specific elevation of non-symbiotic hemoglobin AtHb1: beneficial effects and underlying molecular networks in *Arabidopsis thaliana.* BMC Plant Biol **11:** 48

Tiessen A, Hendriks JHM, Stitt M, Branscheid A, Gibon Y, Farré EM, Geigenberger P (2002) Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: a novel regulatory mechanism linking starch synthesis to the sucrose supply. Plant Cell **14:** 2191–2213

Tovar-Méndez A, Miernyk JA, Randall DD (2003) Regulation of pyruvate dehydrogenase complex activity in plant cells. Eur J Biochem **270:** 1043–1049

Valerio C, Costa A, Marri L, Issakidis-Bourguet E, Pupillo P, Trost P, Sparla F (2011) Thioredoxin-regulated beta-amylase (BAM1) triggers diurnal starch degradation in guard cells, and in mesophyll cells under osmotic stress. J Exp Bot **62:** 545–555

Vandesteene L, López-Galvis L, Vanneste K, Feil R, Maere S, Lammens W, Rolland F, Lunn JE, Avonce N, Beeckman T, Van Dijck P (2012) Expansive evolution of the trehalose-6-phosphate phosphatase gene family in Arabidopsis. Plant Physiol **160:** 884–896

Vandesteene L, Ramon M, Le Roy K, Van Dijck P, Rolland F (2010) A single active trehalose-6-P synthase (TPS) and a family of putative regulatory TPS-like proteins in Arabidopsis. Mol Plant **3:** 406–419

Van Dijck P, Mascorro-Gallardo JO, De Bus M, Royackers K, Iturriaga G, Thevelein JM (2002) Truncation of *Arabidopsis thaliana* and *Selaginella lepidophylla* trehalose-6-phosphate synthase unlocks high catalytic activity and supports high trehalose levels on expression in yeast. Biochem J **366:** 63–71

van Dijken AJH, Schluepmann H, Smeekens SCM (2004) Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. Plant Physiol **135:** 969–977

Van Houtte H, Vandesteene L, López-Galvis L, Lemmens L, Kissel E, Carpentier S, Feil R, Avonce N, Beeckman T, Lunn JE, Van Dijck P (2013) Overexpression of the trehalase gene AtTRE1 leads to increased drought stress tolerance in Arabidopsis and is involved in abscisic acid-induced stomatal closure. Plant Physiol **161:** 1158–1171

Vogel G, Aeschbacher RA, Müller J, Boller T, Wiemken A (1998) Trehalose-6-phosphate phosphatases from *Arabidopsis thaliana*: identification by functional complementation of the yeast *tps2* mutant. Plant J **13:** 673–683

Wahl V, Ponnu J, Schlereth A, Arrivault S, Langenecker T, Franke A, Feil R, Lunn JE, Stitt M, Schmid M (2013) Regulation of flowering by trehalose-6-phosphate signaling in *Arabidopsis thaliana.* Science **339:** 704–707

Wang P, Du Y, Hou YJ, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK (2015) Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1. Proc Natl Acad Sci USA **112:** 613–618

Weiste C, Dröge-Laser W (2014) The *Arabidopsis* transcription factor bZIP11 activates auxin-mediated transcription by recruiting the histone acetylation machinery. Nat Commun **5:** 3883

Wiese A, Elzinga N, Wobbes B, Smeekens S (2005) Sucrose-induced translational repression of plant bZIP-type transcription factors. Biochem Soc Trans **33:** 272–275

Williams B, Njaci I, Moghaddam L, Long H, Dickman MB, Zhang X, Mundree S (2015) Trehalose accumulation triggers autophagy during plant desiccation. PLoS Genet **11:** e1005705

Wilson RA, Jenkinson JM, Gibson RP, Littlechild JA, Wang ZY, Talbot NJ (2007) Tps1 regulates the pentose phosphate pathway, nitrogen metabolism and fungal virulence. EMBO J **26:** 3673–3685

Wingler A, Delatte TL, O'Hara LE, Primavesi LF, Jhurreea D, Paul MJ, Schluepmann H (2012) Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. Plant Physiol **158:** 1241–1251

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ (2007) An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS One **2:** e718

Xiong Y, McCormack M, Li L, Hall Q, Xiang C, Sheen J (2013) Glucose-TOR signalling reprograms the transcriptome and activates meristems. Nature **496:** 181–186

Yadav UP, Ivakov A, Feil R, Duan GY, Walther D, Giavalisco P, Piques M, Carillo P, Hubberten H-M, Stitt M, Lunn JE (2014) The sucrose-Tre6P nexus: specificity and mechanisms of sucrose signalling by trehalose-6-phosphate. J Exp Bot **65:** 1051–1068

Yang L, Xu M, Koo Y, He J, Poethig RS (2013) Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of MIR156A and MIR156C. Elife **2:** e00260

Yu S, Cao L, Zhou CM, Zhang TQ, Lian H, Sun Y, Wu J, Huang J, Wang G, Wang JW (2013) Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. eLife **2:** e00269

Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ (2009) Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. Plant Physiol **149:** 1860–1871

Zhang Z, Deng Y, Song X, Miao M (2015) Trehalose-6-phosphate and SNF1-related protein kinase 1 are involved in the first-fruit inhibition of cucumber. J Plant Physiol **177:** 110–120

Zhao Z, Zhang W, Stanley BA, Assmann SM (2008) Functional proteomics of *Arabidopsis thaliana* guard cells uncovers new stomatal signaling pathways. Plant Cell **20:** 3210–3226