ANNUAL REVIEWS

Annual Review of Plant Biology

The Role of Trehalose 6-Phosphate (Tre6P) in Plant Metabolism and Development

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Annu. Rev. Plant Biol. 2021. 72:737–60

First published as a Review in Advance on January 11, 2021

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-050718-095929

Keywords

embryogenesis, flowering, shoot branching, sucrose, SUCROSE-NON-FERMENTING1-RELATED KINASE1, trehalose 6-phosphate

Abstract

Trehalose 6-phosphate (Tre6P) has a dual function as a signal and homeostatic regulator of sucrose levels in plants. In source leaves, Tre6P regulates the production of sucrose to balance supply with demand for sucrose from growing sink organs. As a signal of sucrose availability, Tre6P influences developmental decisions that will affect future demand for sucrose, such as flowering, embryogenesis, and shoot branching, and links the growth of sink organs to sucrose supply. This involves complex interactions with SUCROSE-NON-FERMENTING1-RELATED KINASE1 that are not yet fully understood. Tre6P synthase, the enzyme that makes Tre6P, plays a key role in the nexus between sucrose and Tre6P, operating in the phloem-loading zone of leaves and potentially generating systemic signals for source-sink coordination. Many plants have large and diverse families of Tre6P phosphatase enzymes that dephosphorylate Tre6P, some of which have non-catalytic functions in plant development.

Contents

1. INTRODUCTION

Trehalose: a nonreducing disaccharide found in fungi, invertebrates, prokaryotes, and plants; involved in osmoregulation, carbon storage and transport, and stress protection

Trehalose 6-phosphate (Tre6P): a phosphorylated intermediate of trehalose biosynthesis that serves as a signal and regulator of sucrose status in plants

Sucrose: a nonreducing disaccharide that is a major product of photosynthesis and the most commonly transported sugar in vascular plants

Trehalose 6-phosphate (Tre6P) is a signal metabolite that regulates sucrose metabolism in plants and links their growth and development to their metabolic status. It is the intermediate in a two-step pathway for the biosynthesis of trehalose (α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside) mediated by Tre6P synthase (TPS; EC 2.4.1.15) and Tre6P phosphatase (TPP; EC 3.1.3.12) (18). This pathway is common in bacteria, and it plays a central role in the carbon metabolism of fungi and many invertebrates, which use trehalose as an osmolyte, storage reserve, transport sugar, and stress protectant. The presence of trehalose in some nonflowering plants was reported over a century ago (3), but for many years it was thought to be unimportant, or even absent, from flowering plants. This view changed about 20 years ago, when genes encoding TPS and TPP enzymes were found in the model plant species *Arabidopsis* (*Arabidopsis thaliana*) (14, 106). *TPS* and *TPP* genes have since been identified in all major plant taxa (6, 7, 62). In parallel, attempts to engineer trehalose metabolism in plants, by expression of heterologous TPS and TPP enzymes from bacteria and yeast, unexpectedly led to abnormal growth and development of the plants, even though they contained only trace amounts of trehalose (82). It was also discovered that *Arabidopsis tps1* null mutants, lacking the predominant TPS enzyme in this species, fail to complete embryogenesis (34). Even when viable *tps1* seeds were obtained by inducible or embryo-specific expression of TPS1 during embryogenesis, the resulting *tps1* plants were severely stunted and did not flower (45, 102). Together, these observations led to the conclusion that the pathway of trehalose biosynthesis is present in all plants and is essential for normal growth and development at all stages in the plant's life cycle.

With only a few exceptions, flowering plants contain barely detectable amounts of trehalose, with levels that are typically 100 to 1,000 times lower than those of more abundant sugars, especially sucrose (19, 64). Sucrose (β-D-fructofuranosyl α-D-glucopyranoside), like trehalose, is a nonreducing disaccharide and in most flowering plants is the major product of photosynthesis and the sugar that is most commonly transported in the phloem from source leaves to growing sink organs, such as roots, flowers, seeds, fruits, and tubers (63). In quantitative terms, trehalose biosynthesis is thus a relatively minor pathway in plant sugar metabolism, so it was initially surprising that disturbance of this pathway led to such severe growth and developmental defects. A key

breakthrough for solving this conundrum was the discovery that expression of bacterial TPS or TPP enzymes in *Arabidopsis* gave rise to strong, but opposite, phenotypes; TPS-expressing plants had small leaves, early flowering, and a bushy growth habit, whereas TPP-expressing plants had large leaves, late flowering, and few shoot branches (89). This led to the conclusion that changes in the level of Tre6P, the intermediate in the pathway, rather than in the level of trehalose itself, are responsible for the aberrant phenotypes when the trehalose biosynthetic pathway is perturbed. Since this discovery, there has been intense interest in elucidating the function of Tre6P to understand how it exerts such a profound influence on plant growth and development. What has emerged is the concept that Tre6P functions primarily as a signal and regulator of sucrose levels in plants (40, 65, 114).

In this review, we begin by exploring the diverse families of TPS and TPP enzymes in plants, highlighting recent advances that shed new light on how the level of Tre6P in plant tissues is controlled. There follows a description of the sucrose:Tre6P nexus model (114), with a critical examination of this model as a basis for interpreting the function of Tre6P in source leaves and sink organs. We then discuss our current understanding of how Tre6P influences some of the key developmental processes in the plant life cycle—flowering, embryogenesis, and shoot branching—linking these to the metabolic status of the plant. Finally, we draw some general conclusions, highlighting gaps in our knowledge and proposing areas to focus on in future research. Due to space constraints, some important and active areas of Tre6P research cannot be covered in depth. These include the roles of Tre6P (and trehalose) in abiotic stress responses and interactions of plants with beneficial and pathogenic microorganisms and the engineering of Tre6P/trehalose metabolism in crop plants to improve their resilience to biotic and abiotic stresses in the field. The reader is referred to previous reviews that cover these topics (35, 46, 64, 82, 83, 92) and some successful examples of engineering Tre6P metabolism for crop improvement (47, 77, 80).

Trehalose-6-phosphate synthase (TPS): an enzyme that catalyzes the synthesis of trehalose 6-phosphate from uridine diphosphate glucose and glucose 6-phosphate

Trehalose-6-phosphate phosphatase (TPP): an enzyme that catalyzes the dephosphorylation of trehalose 6-phosphate to trehalose

Embryogenesis: the development of an embryo, usually resulting from fertilization of an ovule, for example, during seed development

Sink: a tissue or organ that is a net consumer of sugars and other nutrients for growth and the accumulation of storage reserves

Source: a tissue or organ that is a net supplier of sugars, amino acids, and other compounds for other parts of the plant

2. ORIGINS AND EVOLUTION OF TREHALOSE METABOLISM IN PLANTS

TPS and *TPP* genes are present in single-celled chlorophyte algae, streptophyte algae, and all major groups of land plants, indicating that the pathway was already present at the beginning of the green plant lineage (7, 62). Following the initial identification of *TPS* (*AtTPS1*) and *TPP* (*AtTPPA* and *AtTPPB*) genes in *Arabidopsis* (14, 106), the sequencing of the *Arabidopsis* genome revealed these were members of large gene families, with a total of 11 *TPS* genes (*AtTPS1–AtTPS11*) and 10 *TPP* (*AtTPPA–AtTPP7*) genes in this species (53). Phylogenetic analyses revealed that the *TPS* genes divide into two distinct clades: class I (*AtTPS1–AtTPS4*) and class II (*AtTPS5–AtTPS11*) (58) (**Figure 1**). Both clades are represented in chlorophyte algae and throughout the green plant lineage, and both the *TPS* and the *TPP* gene families have expanded independently in different divisions of the plant kingdom (7, 62, 86, 104, 105, 115). The functions of the class I TPS, class II TPS and TPP proteins are discussed in the following sections, along with the potential significance of their diversity.

2.1. Trehalose-6-Phosphate Synthase Class I

In *Arabidopsis*, the class I clade contains four *TPS* genes, three of which—*AtTPS1*, *AtTPS2*, and *AtTPS4*—have been shown to encode catalytically active TPS enzymes based on complementation of the yeast *tps1Δ* mutant (14, 29, 101, 105). *AtTPS3* is most likely a pseudogene (62). To date, none of the class II TPS proteins have been reproducibly shown to complement the yeast *tps1Δ* mutant or have TPS activity in vitro (48, 86, 105). The AtTPS1 protein and its orthologs in other species contain N- and C-terminal domains flanking a central glucosyltransferase domain that

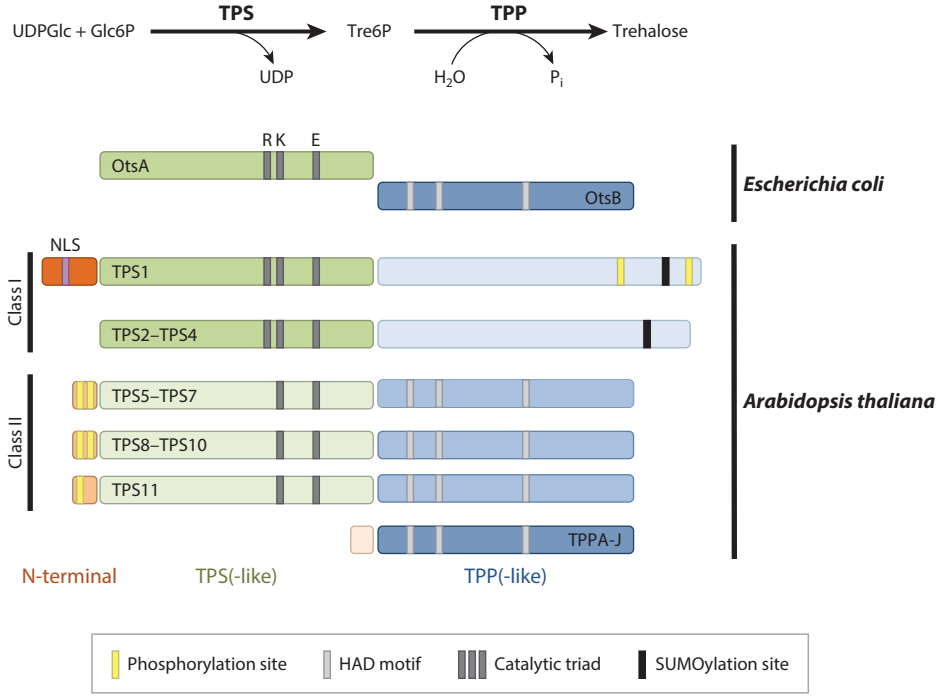

Figure 1

The pathway and enzymes of trehalose biosynthesis. Trehalose-6-phosphate synthase (TPS) catalyzes the synthesis of trehalose 6-phosphate (Tre6P) from uridine diphosphate (UDP)-glucose and glucose 6-phosphate (Glc6P), and then Tre6P is dephosphorylated to trehalose by trehalose-6-phosphate phosphatase (TPP). The *Escherichia coli* TPS (OtsA) and TPP (OtsB) enzymes are single-domain proteins. OtsA contains a catalytic triad of residues—Arg (R), Lys (K), and Glu (E)—that are required for Tre6P synthesis (*dark gray bars*). OtsB is a member of the haloacid dehalogenase (HAD) superfamily of phosphatases and other hydrolases, with three peptide motifs that contain active site residues (*light gray bars*) (62). *Arabidopsis* has 11 *TPS* genes and 10 *TPP* genes (58). The former are subdivided into two main clades: class I (*AtTPS1–AtTPS4*) and class II (*AtTPS5–AtTPS11*). Only the class I proteins have TPS activity (29, 86). AtTPS1 contains three protein domains and is the predominant Tre6P-synthesizing enzyme in *Arabidopsis*. It is targeted mainly to the nucleus by a monopartite nuclear localization signal (NLS) in the N-terminal domain (38). Its C-terminal domain resembles TPP enzymes but has no TPP activity. This domain contains a putative SUMOylation site (*black bar*) and two phosphorylation sites (*yellow bars*) and appears to be essential for catalytic fidelity (38). Two of the remaining class I proteins, AtTPS2 and AtTPS4, also have TPS activity but lack N-terminal domains and are expressed almost exclusively in endosperm tissues in developing seeds (see **Figure 4**). The short forms of class I TPS are found only in the Brassicaceae (62). The class II TPS proteins have TPS- and TPP-like domains and several conserved phosphorylation sites but no known enzymatic activity, and their functions are unknown. All 10 *AtTPP* genes encode catalytically active TPP enzymes (104). In maize (*Zea mays*), at least two TPP enzymes also have noncatalytic moonlighting functions (24).

contains the catalytic site and has similarity with single-domain TPS enzymes in bacteria (e.g., OtsA from *Escherichia coli*) (**Figure 1**).

The functions of the three domains of AtTPS1 have been investigated by complementation of the *Arabidopsis tps1-1* mutant with various constructs based on the genomic sequence of *AtTPS1*, including the native promoter, untranslated regions, and introns, and encoding wild-type, truncated, or mutated versions of AtTPS1 (38). These investigations showed that the

N-terminal domain contains a putative monopartite nuclear localization signal (R_{28}-E-K-R-K_{32}) (**Figure 1**) that targets the majority of the AtTPS1 to the nucleus in various cell types, with the remainder being located in the cytosol. The N-terminal domain also contains a Leu/Arg-rich motif that has been conserved from chlorophyte algae through to flowering plants (6, 7, 62) and has an autoinhibitory effect on the enzyme's activity when expressed in yeast (101). However, disruption of this motif by substitution of Leu_{27} by Pro had little impact on the enzyme's activity in complemented *tps1-1* lines expressing the mutated enzyme (38).

The C-terminal domain has similarity with plant TPP enzymes but lacks some of the residues associated with the active site of TPP enzymes (**Figure 1**), including an Asp residue that plays a central role in catalysis (62) (see Section 2.2). Expression of truncated forms of AtTPS1 lacking the C-terminal domain (AtTPS1[ΔC]) was able to rescue the *tps1-1* mutant through embryogenesis, but the resulting plants were severely stunted and unable to flower (38). Very similar phenotypes were observed when *tps1-1* was complemented with AtTPS1 carrying an A119W point mutation within the catalytic domain (38). In both cases, the plants contained readily detectable levels of two unidentified disaccharide-monophosphates that were either absent or in trace amounts in wild-type plants. As these two molecules were isomers of Tre6P, it was suggested that they were the products of catalytic errors by the AtTPS1[A119W] and AtTPS1[ΔC] forms of AtTPS1 during Tre6P synthesis (38). The levels of these two compounds were lower, and the growth defects less severe, in AtTPS1[ΔNΔC] and AtTPS1[ΔN A119W] lines, in which the N-terminal domain of the AtTPS1 protein had also been removed, indicating functional interactions between the three domains in the wild-type AtTPS1 protein (38).

The C-terminal domain contains two experimentally demonstrated phosphorylation sites (Ser_{827} and Ser_{941}), along with a putative SUMOylation site (Lys_{902}) (**Figure 1**). The latter occurs within a peptide motif—S_{895}·W·N·V·L·D·L·[K^{SUMO}]·G·E·N·Y·F·S·C_{909}—that matches the SUMOylation site consensus sequence and is highly conserved in class I TPS enzymes in all the major land plant groups and streptophyte algae (38). Complementation of *tps1-1* by expression of AtTPS1 with a 48-amino-acid truncation at the C terminus (TPS1[$\Delta C_{895-942}$]) gave rise to plants with very similar phenotypes to the AtTPS[ΔC] lines, including elevated levels of the two unknown disaccharide-monophosphates. Thus, much of the functionality of the C-terminal domain of AtTPS1 appears to be associated with the putative SUMOylation site and distal phosphorylation site (Ser_{941}), suggesting that posttranslational modifications at these sites are important for regulating the enzyme's activity (38).

In contrast to *AtTPS1*, which is expressed in all major organs of the plant, expression of the other two functional class I genes in *Arabidopsis*, *AtTPS2* and *AtTPS4*, is largely restricted to specific tissues within developing seeds (40, 105). The *AtTPS2* and *AtTPS3* loci are adjacent on chromosome 1 in the *Arabidopsis* genome, and this tandem repeat lies within a region of conserved synteny with the *AtTPS1* locus, indicating that *AtTPS2* and *AtTPS3* arose via a segmental duplication (62). The origin of the *AtTPS4* gene is less clear. The AtTPS2 and AtTPS4 proteins share similar glucosyltransferase and C-terminal domains with AtTPS1 but lack an N-terminal domain (**Figure 1**). When heterologously expressed in yeast, they appear to have higher enzymatic activity than AtTPS1 (29), consistent with the absence of the Leu/Arg-rich autoinhibitory motif. Such short forms of class I TPS proteins have so far only been found in members of the Brassicaceae (62). The significance of this limited distribution is unknown. The potential function of these short class I TPS proteins during embryogenesis is discussed in Section 4.2.

2.2. Trehalose-6-Phosphate Synthase Class II

Phylogenetic studies indicate that the class II TPS proteins in *Arabidopsis* and other flowering plants can be divided into at least two separate clades, with AtTPS5–AtTPS7 clustering

separately from AtTPS8–AtTPS10 (51, 62, 115). The phylogenetic relationship of AtTPS11 and its orthologs is less clear, with some studies placing them on a deep-rooted branch of the AtTPS8–AtTPS10 clade, while others put them in a separate clade of their own. The class II TPS proteins resemble those of the class I in having a glucosyltransferase-like domain and a C-terminal TPP-like domain, but they lack an N-terminal domain (**Figure 1**). Their glucosyltransferase-like domain contains some but not all of the active site residues (**Figure 1**), and they are unable to complement, reproducibly, the yeast *tps1Δ* mutant (86, 105). The TPP-like domains of the class II TPS proteins contain three peptide motifs that are associated with the active site of TPP enzymes and characteristic of all members of the haloacid dehalogenase (HAD) superfamily of proteins (62) (**Figure 1**). Despite the high conservation of TPP active site residues, none of the class II TPS proteins have been demonstrated to have TPP activity, either by complementation of the TPP-deficient yeast *tps2Δ* mutant or by in vitro assay of recombinant proteins (48, 86, 107). In the absence of demonstrable TPS or TPP activities, the functions of the class II TPS proteins remain largely a mystery nearly 20 years after this enigmatic family of proteins was first described (58).

Abscisic acid (ABA): a phytohormone involved in responses to environmental stresses and in regulation of plant development, stomatal conductance, and seed/bud dormancy

Loss-of-function mutants and overexpression studies have implicated class II TPS proteins in responses to abscisic acid (ABA) signaling (TPS5) (98), thermotolerance (TPS5) (96), basal pathogen defense (TPS5) (109), the regulation of cell shape (TPS6) (20), cold tolerance (TPS11) (61), and aphid resistance (TPS11) (91). However, details of the molecular mechanisms involved remain sketchy. Molecular functions that have been proposed for the class II TPS proteins include (*a*) the regulation of class I TPS enzyme activity, based on the similarity of these proteins to noncatalytic subunits of the trehalose-synthesizing complex in yeast (62) and the association of rice class I and II proteins in yeast two-hybrid and bimolecular fluorescence complementation assays (116); (*b*) signaling proteins, based on the conservation of ligand-binding site residues in their glucosyltransferase-like and TPP-like domains, giving them the potential to bind Tre6P and other related molecules (62). It has also been noted that the class II TPS proteins have some resemblance to the bifunctional synthase-phosphatase enzymes that are responsible for the synthesis of glucosylglycerol in cyanobacteria (67) and floridoside and isofloridoside in red algae (Rhodophyta) (81) via phosphorylated intermediates. These heterosidic compounds are rare in flowering plants and have not been reported in *Arabidopsis*. Nevertheless, the possibility that the class II TPS proteins do have catalytic activity and are involved in synthesizing some kind of disaccharide, other than trehalose, has not yet been definitively excluded.

2.3. Trehalose-6-Phosphate Phosphatase

There are 10 *TPP* genes in *Arabidopsis* (**Figure 1**), all of which encode catalytically active TPP enzymes based on their ability to complement the yeast *tps2Δ* mutant (104, 106). This large gene family arose via repeated genome duplication events, and 8 out of the 10 genes are paralogous pairs: *AtTPPB* and *AtTPPC*, *AtTPPE* and *AtTPPH*, *AtTPPF* and *AtTPPG*, and *AtTPPI* and *AtTPPJ* (104). Such a high degree of paralog retention is unusual for enzyme genes and is more often seen in transcription factor and other regulatory protein gene families. Promoter analysis with β-GLUCURONIDASE (GUS) and GREEN FLUORESCENT PROTEIN (GFP) reporters revealed that the 10 *AtTPP* genes have different spatiotemporal expression patterns, indicating neofunctionalization after gene duplications (104). The AtTPP proteins also differ in their subcellular compartmentation, with some being localized in chloroplasts (AtTPPD and AtTPPE), the nucleus (AtTPPG), or both nuclei and peroxisomes (AtTPPI), and the remainder being cytosolic (52, 54).

Several *TPP* genes have been linked to abiotic stress responses. In rice, *OsTPP1* and *OsTPP2* are induced by cold stress (84, 90), while the *OsTPP7* gene confers resistance to anaerobiosis during

germination, a useful trait that has been lost, along with the *OsTPP7* gene, from many commercial cultivars (55). Expression of the *OsTPP1* gene in developing maize ears under the control of the *OsMAD6* promoter improved yield under drought conditions by preventing kernel abortion, with no yield penalty under well-watered conditions (77). This was linked to changes in expression of *SWEET* (*SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTER*) genes encoding sucrose efflux carriers and a shift in sucrose allocation to the seeds (80). In *Arabidopsis*, a redox-regulated plastidial isoform, AtTPPD, has been linked to salt and oxidative stress resistance (54), and two other isoforms, AtTPPF and AtTPPI, are involved in responses to drought (59, 60).

Several TPP proteins have also been shown to play significant roles in plant development. In maize (*Zea mays*), the *RAMOSA3* (*RA3*) gene is expressed in localized regions of inflorescence primordia and encodes a catalytically active TPP. Loss-of-function *ra3* mutants show increased branching of both the ears and tassels (88), although there were no significant differences in Tre6P or trehalose levels compared to wild-type primordia (19). A screen for enhancer mutants in the *ra3* mutant background identified four allelic mutants with similarly increased inflorescence branching, all of which had lesions in the *ZmTPP4* gene (24). The *ZmTPP4* gene was upregulated in the *ra3* mutant, has a similar expression pattern to *RA3* in inflorescence primordia, and also encodes a catalytically active TPP (24). However, there were no significant differences in Tre6P levels between wild-type, *ra3*, and *ra3 tpp4* inflorescence primordia. Among the four *ZmTPP4* allelic variants, some retained up to 40% of wild-type TPP activity, while others were catalytically inactive, yet all of the *ra3 tpp4* double mutants had essentially the same degree of inflorescence branching (24). Together, these results suggested that the branching phenotype was independent of the loss of TPP catalytic activity in the mutants. This was confirmed by the demonstration that a mutated (D110E), catalytically inactive form of RA3 expressed under the control of the *RA3* promoter could substantially complement the *ra3* mutant (24). Researchers concluded that the branching defects in the *ra3* and *ra3 tpp4* mutants are independent of the catalytic activities of RA3 and ZmTPP4 and that these two proteins have noncatalytic moonlighting functions in the regulation of maize inflorescence development (24). Potential signaling functions might involve binding of Tre6P in a noncatalytic conformation or binding of other ligands and interactions with other proteins. A connection between TPP and auxin signaling has been observed in *Arabidopsis tppi* knockdown mutants, with auxin movement by PIN1 and PIN3 auxin efflux carriers being compromised, leading to restriction of primary root growth (59). The roles of Tre6P and trehalose metabolic enzymes in plant development are discussed further in Section 4.

SWEET (SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTER): sugar efflux carrier proteins involved in the movement of sucrose and other sugars across the plasmalemma and tonoplast membranes

Auxin: a class of phytohormone (most commonly indole-3-acetic acid) involved in regulation of plant growth and development

Shoot apical meristem (SAM): tissue containing undifferentiated cells, including stem cells, at the shoot tip

3. THE SUCROSE:TREHALOSE 6-PHOSPHATE NEXUS

A recurring theme in studies of Tre6P in plants is a connection with sucrose, the dominant sugar in vascular plants. When Tre6P was first identified as a molecule of interest in plants, researchers proposed that it played a role in the regulation of sucrose utilization (89). Mass spectrometric methods that were sensitive and specific enough to measure the very low amounts of Tre6P in plant tissues revealed that the level of Tre6P in *Arabidopsis* seedlings and rosettes is highly correlated with that of sucrose (65). The level of Tre6P is highly dynamic, with up to 40-fold increases in Tre6P content observed when sucrose was supplied to carbon-starved *Arabidopsis* seedlings (65, 78, 114). Tre6P also changes in response to endogenous fluctuations in sucrose levels, for example, during the diel light-dark cycle in leaves, and these two metabolites were highly correlated in *Arabidopsis* rosettes across a wide range of growth conditions (2, 19, 31, 39, 65, 69, 95, 112). Correlations between sucrose and Tre6P levels have also been observed in other *Arabidopsis* tissues, including the shoot apical meristem (SAM) (108), and in other species, including potato (*Solanum tuberosum*) (27), wheat (*Triticum aestivum*) (68), maize (51), and cucumber (*Cucumis sativa*) (119).

The responsiveness of Tre6P to sucrose appears to be specific, with responses to changes in the levels of other sugars, such as glucose and fructose, or nitrogen supply explained by concomitant changes in sucrose levels (114). In *Arabidopsis* seedlings, the kinetics of the Tre6P response and differential sensitivity to various inhibitors showed that the rise in Tre6P after sucrose feeding was not simply due to mass action effects, i.e., the increased availability of the substrates for Tre6P synthesis: Glc6P and uridine diphosphate glucose (114). The dynamic responsiveness of Tre6P to changes in sucrose levels led to the proposal that Tre6P might function as a signal metabolite, reporting the availability of sucrose (65) as well as regulating its utilization for growth (89).

Another intriguing observation was that sucrose levels change in the opposite direction to Tre6P when Tre6P levels are manipulated by overexpression of TPS or TPP (114). In an attempt to provide a conceptual framework for understanding these various findings, the sucrose:Tre6P nexus model was proposed. This model postulates that Tre6P has a dual function as both a signal and a negative feedback regulator of sucrose levels (40, 114). Researchers hypothesize that as Tre6P follows the diel fluctuations in sucrose in source leaves it acts to lower sucrose production if the levels of sucrose in the leaves rise too high and to stimulate sucrose production if levels fall too low (40) (**Figure 2**). Such homeostatic regulation of sucrose has been likened to the function of insulin in the control of blood glucose levels in animals (40, 114). Researchers envisage the sucrose-signaling function of Tre6P coming to the fore in sink organs, with high Tre6P being a sign that sucrose supplies are abundant and low Tre6P indicating that supplies are limited. By signaling sucrose availability, Tre6P provides information about the metabolic status of the plant that can be integrated with other endogenous signals (e.g., phytohormones) and environmental cues to bring about appropriate developmental responses. These responses include a commitment to flowering, growth of new shoot branches, and seed production, all of which have a significant impact on the carbon budget of the plant in the future. According to this model, once a developmental commitment has been made, Tre6P continues to play a role in monitoring sucrose supply to the growing sink organ and regulating its utilization of sucrose for growth accordingly.

Another feature of the nexus relationship between sucrose and Tre6P in this model is its flexibility, with the sensitivity and response range of Tre6P being adjusted to suit the particular needs of individual tissues and developmental stages. Evidence for this flexibility comes from a comparison of leaves and shoot apices; in each tissue, Tre6P is highly correlated with sucrose, but the sucrose:Tre6P ratio in leaves is tenfold higher than in shoot apices (19, 64, 69, 108). The nexus can also respond to changes in environmental conditions, as indicated by the fivefold-higher sucrose:Tre6P ratio in leaves of cold-grown (8°C) *Arabidopsis* plants than in plants grown at 20°C, with Tre6P closely tracking the diel fluctuations in sucrose levels at both growth temperatures (19). Such adaptation of the nexus can explain why in some circumstances the sucrose-Tre6P relationship appears to break down. For example, in grapevine (*Vitis vinifera*), there were huge changes in both the sucrose and Tre6P contents of the berries at different stages of development, but the levels of these two metabolites were poorly correlated if compared across the whole 2-month period of fruit development and ripening (26). Similarly, the correlations between sucrose and Tre6P levels in developing wheat and maize seeds were also weak when metabolite levels were compared over the whole course of seed development (50, 68). At first sight, these findings seem to contradict the nexus model. However, it must be remembered there are major developmental changes in growing tissues over these long timescales, with transitions from cell division to cell expansion, followed by accumulation of storage reserves and then fruit ripening and seed maturation. It is well established that there are major adjustments in metabolism during these developmental transitions, so it should not be surprising if the relationship between sucrose and Tre6P were also adjusted to new settings that are appropriate for that particular stage of development. Thus, comparing sink organs at different developmental stages could easily lead to the

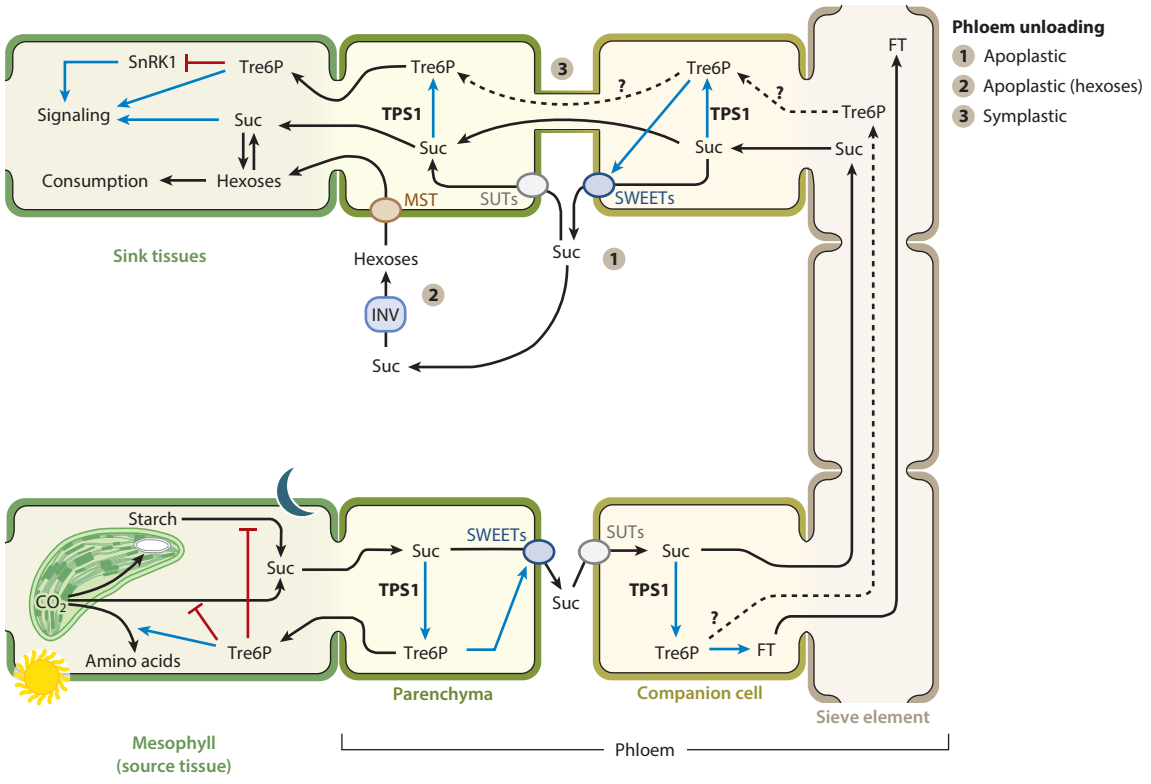

Figure 2

The sucrose:Tre6P nexus in source leaves and sink tissues. Tre6P acts as both a signal and regulator of Suc levels in plants (40, 114). In *Arabidopsis* source leaves, Tre6P made by AtTPS1 in the phloem parenchyma of the bundle sheath can move symplastically into mesophyll cells, where it regulates photosynthetic sucrose production during the day and the remobilization of transitory starch reserves to sucrose at night to balance supply with demand for sucrose from growing sink organs (39, 69). Tre6P is also made in the phloem companion cells, potentially generating long-distance signals involved in source-sink communication, including sucrose, FT (a phloem-mobile protein), and possibly Tre6P itself (36). Tre6P potentially regulates apoplastic phloem unloading by modulating the expression of SWEET sucrose efflux carriers. In sink tissues, Tre6P regulates the utilization of sucrose for growth and accumulation of storage reserves (89), in part via inhibition of SnRK1 (9, 117, 118). Black lines represent metabolic fluxes and transport processes, with dashed lines indicating putative transport paths, and blue and red lines represent positive and negative interactions, respectively. Abbreviations: FT, FLOWERING LOCUS T; INV, invertase; MST, monosaccharide transporter; SnRK1, SUCROSE-NON-FERMENTING1-RELATED KINASE1; Suc, sucrose; SUT, sucrose-H^+ symporter; SWEET, SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTER; TPS1, trehalose-6-phosphate synthase 1; Tre6P, trehalose 6-phosphate.

conclusion that the relationship has broken down, when in reality it has simply been adjusted to a new state. Differences in timescale probably explain why strong correlations between sucrose and Tre6P are reproducibly observed in leaves and seedlings over relatively short time periods (minutes to hours), but correlations have not always been seen in experiments over longer time frames (e.g., seed and fruit development) when sampling intervals are typically measured in days or weeks and major metabolic and developmental changes are occurring.

The sucrose:Tre6P nexus model is continually evolving, and some or even all aspects of the model may eventually be rejected as we improve our understanding of Tre6P and its functions in plants. Nevertheless, for the moment, we consider that it provides a plausible explanation for most of the phenotypes reported from plants with altered Tre6P levels and is a useful framework

for formulating hypotheses and designing experiments to investigate the functions of Tre6P in plants.

Photoassimilate partitioning: the allocation of the intermediates of photosynthetic carbon fixation for the synthesis of sucrose, starch, and other end products in leaves

Transitory starch: a glucose-containing polysaccharide synthesized in the leaves during the day and degraded at night to supply sugars to the plant

3.1. Source Leaves

Constitutive overexpression of TPS or TPP was the key to recognizing the importance of Tre6P in plants (89), but such plants have limitations, as the phenotypes are so pleiotropic that it can be difficult to disentangle primary and secondary effects. Later studies using chemically inducible promoters to drive TPS expression (39, 69), or pharmacological approaches with membrane-permeable analogs of Tre6P (47), allowed the impact of short-term increases in Tre6P to be investigated. In *Arabidopsis* leaves, an induced rise in Tre6P led to a shift in photoassimilate partitioning, with less carbon going into sucrose synthesis and more being used for organic and amino acid synthesis (39). This shift was brought about by posttranslational activation of phospho*enol*pyruvate carboxylase, increasing anaplerotic flux of carbon into the tricarboxylic acid cycle, and posttranslational activation of nitrate reductase (39). Together, these changes will increase the supply of reduced nitrogen and carbon skeletons, both of which are needed for amino acid synthesis. At night, an induced rise in Tre6P inhibited the remobilization of transitory starch reserves to sucrose in leaves (69). The inhibition appears to operate at an early step in the pathway of starch breakdown in the chloroplasts and is integrated with the regulation of this process by the circadian clock, but the molecular mechanism has not yet been elucidated (31, 69). In this context, it is worth noting that modulation of the clock by sugars is dependent on AtTPS1 (41).

Complementation of the *Arabidopsis tps1-1* mutant with GUS- or GFP-tagged AtTPS1 showed that in leaves the protein is located primarily in and around the vascular tissue, especially the companion cell-sieve element complex; around the phloem parenchyma of the bundle sheath; and in stomatal guard cells (38) (**Figure 2**). Previous studies on *Arabidopsis tps1*, *tppg*, and *trehalose1* mutants revealed the importance of Tre6P and trehalose metabolism for the regulation of stomatal conductance, including sensitivity to ABA (45, 103, 104). The potential functions of Tre6P in stomata were reviewed in Reference 40.

Arabidopsis is an apoplastic phloem loader, and the presence of AtTPS1 in cells on either side of the apoplastic barrier places it in strategic sites at the interface between source and sink tissues (**Figure 2**). The phloem parenchyma cells of the bundle sheath are symplastically connected, via plasmodesmata, with the mesophyll cells where sucrose is produced and are responsible for the movement of sucrose into the apoplast via SWEET sucrose efflux carriers (21). If the production of sucrose in the leaves exceeds the transport capacity of the phloem or demand from growing sink organs, it will accumulate in the phloem parenchyma and mesophyll cells, triggering a rise in Tre6P made by AtTPS1 in the phloem parenchyma. Tre6P will be able to diffuse symplastically into the mesophyll cells, where it can divert photoassimilates away from sucrose toward organic and amino acids during the day or slow down the remobilization of transitory starch reserves at night (**Figure 2**). Conversely, if sucrose production is too slow to meet demand, sucrose and Tre6P levels will fall, allowing more photoassimilates to be directed towards sucrose synthesis in the light or faster turnover of starch reserves at night. Such homeostatic regulation of sucrose production by Tre6P in leaves helps the plant to balance the supply of sucrose with demand. Leaves also export amino acids, alongside sucrose, via the phloem, and Tre6P might also help to ensure that the supplies of sucrose and amino acids from the leaves are properly matched to meet both the carbon and nitrogen needs of growing sink tissues (39, 40).

The molecular mechanisms by which Tre6P responds to fluctuations in sucrose are still poorly understood. Inhibitor studies indicated that in *Arabidopsis* seedlings the Tre6P response to exogenous sucrose supply is dependent on de novo translation but not transcription (114). More

recently, complementation studies with the *Arabidopsis tps1-1* mutant showed that AtTPS1 plays an important role in connecting Tre6P with sucrose (38). In most of the complemented lines expressing wild-type or modified forms of AtTPS1, Tre6P levels in rosettes were highly correlated with sucrose (38). Expression of the *E. coli* TPS (OtsA), under the control of the *AtTPS1* promoter and other potential gene regulatory elements, rescued the *tps1-1* mutant through embryogenesis (38). Root growth was impaired in the OtsA-complemented plants, but otherwise their growth and development were very similar to that of wild-type plants (38). Although the OtsA-complemented plants had Tre6P and sucrose levels in a similar range as those in wild-type plants, Tre6P and sucrose were poorly correlated, indicating that AtTPS1 is important for tying Tre6P to sucrose. This result also appears to question the physiological importance of the relationship between sucrose and Tre6P, at least in source leaves. However, photoassimilate partitioning is regulated by a complex network of transcriptional, posttranslational, and allosteric mechanisms (94). The near wild-type metabolite levels in the OtsA-complemented *tps1-1* plants indicate that some of these other mechanisms were indeed still operating to regulate sucrose levels, even though the sucrose:Tre6P nexus mechanism appeared to be broken. Such robustness is a common feature of many metabolic networks, and there could be sufficient redundancy within the network to allow the OtsA-complemented plants to grow relatively normally, at least under standard laboratory conditions. It remains to be tested whether disruption of the nexus in these plants has a more severe effect when the plants are grown under suboptimal conditions.

SUCROSE-NON-FERMENTING1-RELATED KINASE1 (SnRK1): a trimeric protein kinase complex involved in regulation of plant growth and stress responses

In addition to the leaves, sucrose can also be provided by remobilization of short- and long-term storage reserves (e.g., starch, fructans, and oil) in germinating seeds and in sprouting bulbs and tubers. Stem reserves of starch or fructans can make a major contribution to grain filling in cereals, and many perennial woody plants accumulate starch reserves in their stems or roots during the growing season to fuel springtime growth. It seems likely that Tre6P is involved in the management of such reserves and their remobilization to supply sucrose, but so far there have been few studies in this area.

3.2. Sink Organs

Nonphotosynthetic organs, such as roots, flowers, developing seeds, and tubers, are dependent on the supply of sucrose from source leaves for the carbon and energy they need for growth and accumulation of storage reserves (63). Here, the sucrose-signaling function of Tre6P that regulates sucrose utilization is the dominant aspect of the sucrose:Tre6P relationship (40, 89) (**Figure 2**). Researchers propose that in meristematic and dormant tissues Tre6P provides information about the plant's capacity to supply sucrose, influencing developmental decisions, e.g., flowering and shoot branching (**Figure 3**), that will create increased demand for sucrose in the future (see Section 4). The growth that follows developmental transitions is also regulated by Tre6P to ensure that it matches the availability of sucrose from the leaves and other source tissues. Two protein kinase complexes, SUCROSE-NON-FERMENTING1-RELATED KINASE1 (SnRK1) and TARGET OF RAPAMYCIN (TOR), are central hubs in metabolic regulation and act antagonistically on growth, with SnRK1 repressing and TOR activating growth (8). In *Arabidopsis*, expression of genes encoding subunits of the TOR or SnRK1 complexes overlaps with that of *AtTPS1* in the leaf and root vasculature, indicating that interactions between TOR, SnRK1, and AtTPS1/Tre6P could be occurring in these tissues (38, 72, 111). At present, we have no direct evidence of regulation of TOR by Tre6P. However, *Arabidopsis lst8* mutants, lacking a regulatory subunit of the TOR complex, have similar metabolic profiles (72) to plants with elevated Tre6P levels (114), suggesting a possible connection between TOR and Tre6P. In contrast, connections between Tre6P and SnRK1 have been more extensively studied. This topic has been recently reviewed (9), so here we highlight only some key points.

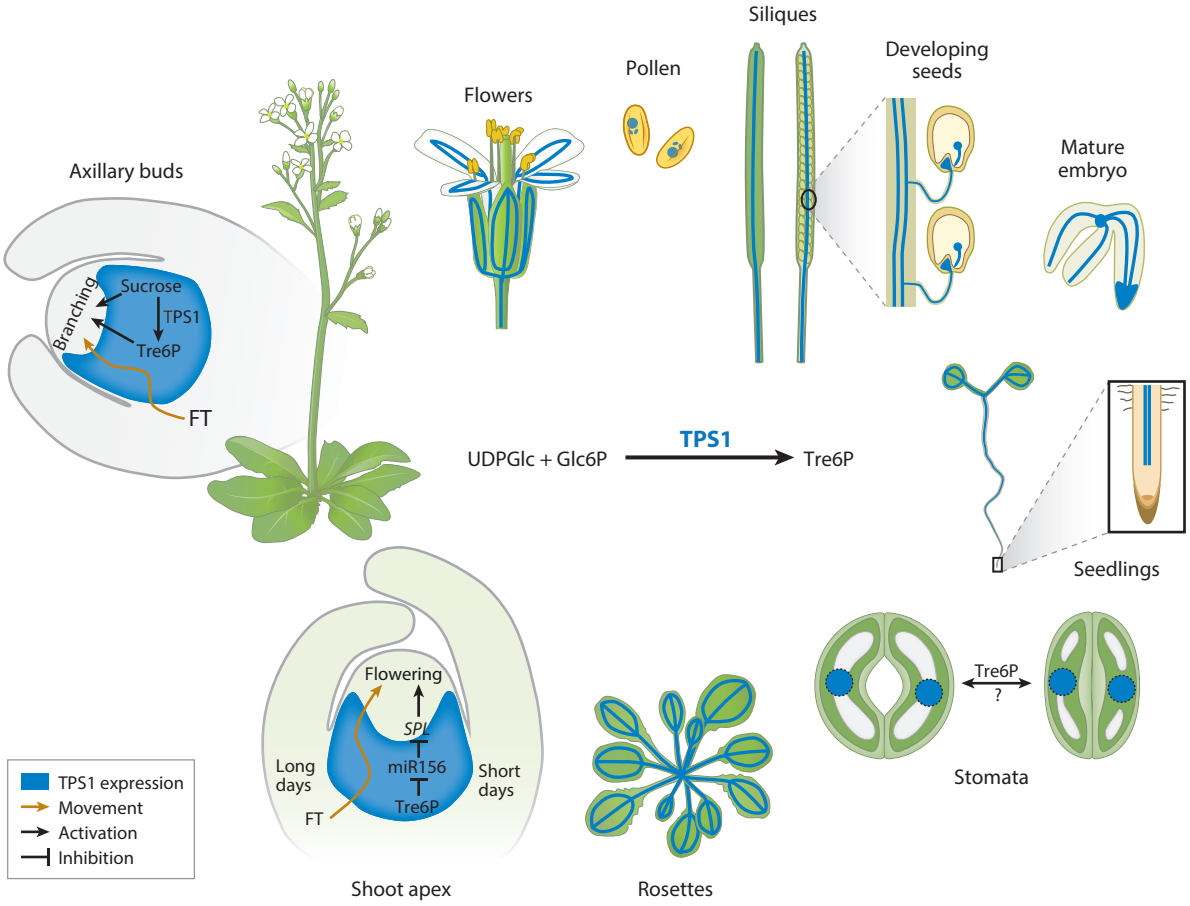

Figure 3

Localization of *Arabidopsis* AtTPS1 and regulation of developmental transitions by Tre6P. AtTPS1 is the predominant Tre6P-synthesizing enzyme in *Arabidopsis*. By complementation of a *tps1* mutant with tagged forms of the protein, AtTPS1 was found to be located in the vasculature of leaves, roots, floral tissues, and siliques; in stomatal guard cells in leaves and flowers; in mature pollen grains; and in the embryo and funiculus of developing seeds (38). It is also located in the rib and peripheral regions of the shoot apical meristem and in axillary buds (38). Tre6P regulates flowering time via modulation of *FT* expression in leaves (long days) and via interactions with the age-dependent pathway involving miR156 and SPL proteins (108). Tre6P is essential for embryogenesis (34, 38) and regulates the outgrowth of rosette axillary buds into new shoots (36, 37). Brown arrows represent transport processes. Abbreviations: *FT*, *FLOWERING LOCUS T*; Glc6P, glucose 6-phosphate; miR156, microRNA 156; SPL, SQUAMOSA PROMOTER BINDING PROTEIN LIKE; TPS1, trehalose-6-phosphate synthase 1; Tre6P, trehalose 6-phosphate; UDPGlc, uridine diphosphate α-D-glucose.

Several of the *Arabidopsis* class II TPS proteins are potential targets for phosphorylation by SnRK1 (43, 48) (**Figure 1**), but the impact of this on Tre6P metabolism and function in vivo, if any, is not yet understood. Suppression of SnRK1 in developing pea (*Pisum sativum*) seeds led to developmental stage–dependent changes in expression of the class I *TPS* gene (*PsTPS1*), down-regulation of class II *TPS* genes (*PsTPS5* and *PsTPS9*), and increased levels of sucrose and Tre6P in the seeds (85). More is known about Tre6P-SnRK1 interactions in the opposite sense, i.e., the impact of Tre6P on SnRK1. It was shown that Tre6P can inhibit SnRK1 in developing *Arabidopsis* tissues, with inhibition being dependent on a heat-labile factor that is present in young,

but not older, leaves (118). Inhibitory effects of Tre6P on SnRK1 have since been reported from other growing tissues (e.g., seedlings) (78, 79) and in other species (e.g., developing potato tubers) (27). The inhibition of SnRK1 activity by Tre6P leads to changes in gene expression, mediated, at least in part, via modulation of the bZIP11 transcription factor (25, 28, 66). A second mechanism of SnRK1 regulation by Tre6P involves direct binding of Tre6P to the catalytic subunit (SnRK1α) of the SnRK1 complex (117). This inhibits interaction of the SnRK1 complex with two SnRK1-activating kinases [SNAK1 and SNAK2, also known as GEMINIVIRUS REP-INTERACTING KINASE1 (GRIK1) and GRIK2], thereby lowering SnRK1 activity in vivo (117). Conversely, it was also observed that Tre6P activates SnRK1 activity when assayed in vitro in the absence of SNAKs (117). It should also be noted that, although generally considered to be a repressor of growth, SnRK1 is also essential for growth (9). As discussed in the following sections, Tre6P might also be influencing the metabolism and growth of sink organs via other mechanisms, including interactions with phytohormone signaling and modulation of sucrose transport. The emerging picture is one of multifaceted interactions between Tre6P and SnRK1, embedded within a wider network of regulatory mechanisms that coordinate plant growth with metabolic status (9).

FLOWERING LOCUS T (FT): a florigenic protein that moves from the leaves to the shoot apex, via the phloem, to trigger flowering

4. TREHALOSE 6-PHOSPHATE AND PLANT DEVELOPMENT

The life cycle of flowering plants, from germination to seed production, involves developmental transitions that generally commit the plant to a new growth phase that will consume sucrose and other resources. Developmental transitions that occur in most flowering plants include seed germination, the juvenile-to-adult transition in leaves, flowering, shoot and root branching, and, finally, seed set and fruit production. Other more specific examples include tuberization; sprouting in bulbs, corms, and tubers; and breaking of winter or dry-season dormancy in woody perennials. Tre6P has been implicated as a regulatory factor in several developmental transitions, potentially providing information on whether the plant's capacity to supply sucrose is sufficient for the new growth that will follow the transition. In the following sections, we describe our current understanding of how Tre6P influences some of the key developmental transitions in *Arabidopsis*: flowering, embryogenesis, and shoot branching.

4.1. Flowering

A connection between Tre6P and flowering time was established by the precocious or delayed flowering when TPS or TPP, respectively, was overexpressed in *Arabidopsis* (89). Flowering time is controlled by multiple interacting pathways involving environmental inputs (e.g., photoperiod, temperature, and nutrient availability) and endogenous cues (e.g., phytohormones and the age of the plant). FLOWERING LOCUS T (FT) is a phloem-mobile protein that is synthesized under long-day conditions in the companion cells of the leaf vasculature (**Figure 2**) and moves to the SAM, where it triggers the floral transition (100). Expression of *FT* is under the control of the *CONSTANS* (*CO*) gene, and FT shares some of its functions with a close homolog, TWIN SISTER OF FT (TSF). Using a reverse genetic approach, researchers found that expression of *FT* in *Arabidopsis* leaves is dependent on AtTPS1 (108), although a strict dependence on Tre6P was not formally demonstrated. When the *tps1-1* mutant was complemented by expression of the *E. coli* TPS (OtsA), the complemented plants flowered at the same chronological age and leaf number as wild-type and AtTPS1-complemented plants when grown under long-day conditions (38). This observation definitively showed that the dependence of *FT* expression on AtTPS1 is due to its Tre6P-synthesizing activity, rather than any putative noncatalytic functions (38).

As noted previously, AtTPS1 is predominantly located in the vascular tissue of the leaves, especially in companion cells (38), where *FT* is expressed (**Figure 2**). Localized overexpression of TPS (OtsA) in the vasculature, under the control of the *Flaveria trinervia GLDPA* gene promoter (4), led to higher Tre6P levels; increased expression of *CO*, *FT*, and *TSF*; and early flowering, whereas expression of a heterologous TPP to lower Tre6P in the vasculature delayed flowering (36). These flowering phenotypes resembled those of constitutive TPS- and TPP-overexpressing plants (89) and indicated that the leaf vasculature is the major site of action of Tre6P in the photoperiodic induction of flowering via *FT* (36). In general, high Tre6P levels are associated with early flowering in *Arabidopsis*. However, when the *tps1-1* mutant was partially complemented by expression of some of the mutated (AtTPS1[A119W]) or truncated (AtTPS1[ΔC]) forms of AtTPS1, the plants never flowered, even though they had up to four times more Tre6P than wild-type plants (38). The AtTPS1[A119W]- and AtTPS1[ΔC]-complemented plants contained elevated levels of two unidentified compounds that were isomeric with Tre6P, and it was proposed that these were interfering with Tre6P signaling, thereby preventing the usual flowering response to elevated Tre6P (38).

Using reverse genetic approaches, AtTPS1 was also shown to affect flowering under short-day conditions via interactions with the age-related pathway (108), in which age-dependent decay in the levels of microRNA 156 (miR156) modulates expression of several *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) genes in the SAM (**Figure 3**). The molecular interactions connecting AtTPS1/Tre6P to this pathway in the SAM remain to be elucidated. In situ RNA hybridization showed that *AtTPS1* is expressed around the central zone of vegetative meristems and in the floral primordia of inflorescence meristems (108), and the AtTPS1 protein was located in the peripheral and rib zones of the SAM, where cell division, differentiation, and expansion occur (38). AtTPS1 has been reported to bind to two proteins associated with the cell cycle—cyclin-dependent kinase A:1 (CDKA:1) and a CDKA:1-interacting kinesin—in yeast two-hybrid binding assays (42). AtTPS1 is also present in the protovasculature subtending the SAM and in leaf primordia (38). Together, these findings suggest a potential role for AtTPS1 (and Tre6P) in the regulation of cell division and vascular development. AtTPS1 was also found in several floral tissues, including the vasculature in stamens (filaments and anthers), the guard cells of the anthers, and the nuclei of mature pollen grains (38) (**Figure 3**). The functions of AtTPS1 (and Tre6P) in these male reproductive cell tissues are not yet known. In the next section, we discuss the potential functions of AtTPS1 and Tre6P in maternal and filial tissues after fertilization.

4.2. Embryogenesis

The arrest of *Arabidopsis tps1* embryos at the torpedo stage was one of the earliest observations to show the importance of the trehalose metabolic pathway in plant development (34). Compared to wild-type, the arrested *tps1* embryos had fewer cells; abnormal cell walls; and higher levels of sucrose, starch, and hexoses, but lower levels of storage lipids and proteins (44). Chemically induced expression of *AtTPS1* during seed development allowed the *tps1* mutant embryos to complete embryogenesis and yield viable seeds (102), as did embryo-specific expression of *AtTPS1* under the control of the *ABA INSENSITIVE3* promoter (45). Complementation of the *tps1* mutant by AtTPS1 expression was shown to be dependent on its catalytic activity, and AtTPS1 could be substituted by expression of the *E. coli* TPS (OtsA) under the control of the *AtTPS1* promoter (38), showing that the restoration of Tre6P synthesis in the *tps1* mutant is both necessary and sufficient for it to complete embryo development and showing the dependence of embryogenesis on Tre6P, rather than on AtTPS1 per se.

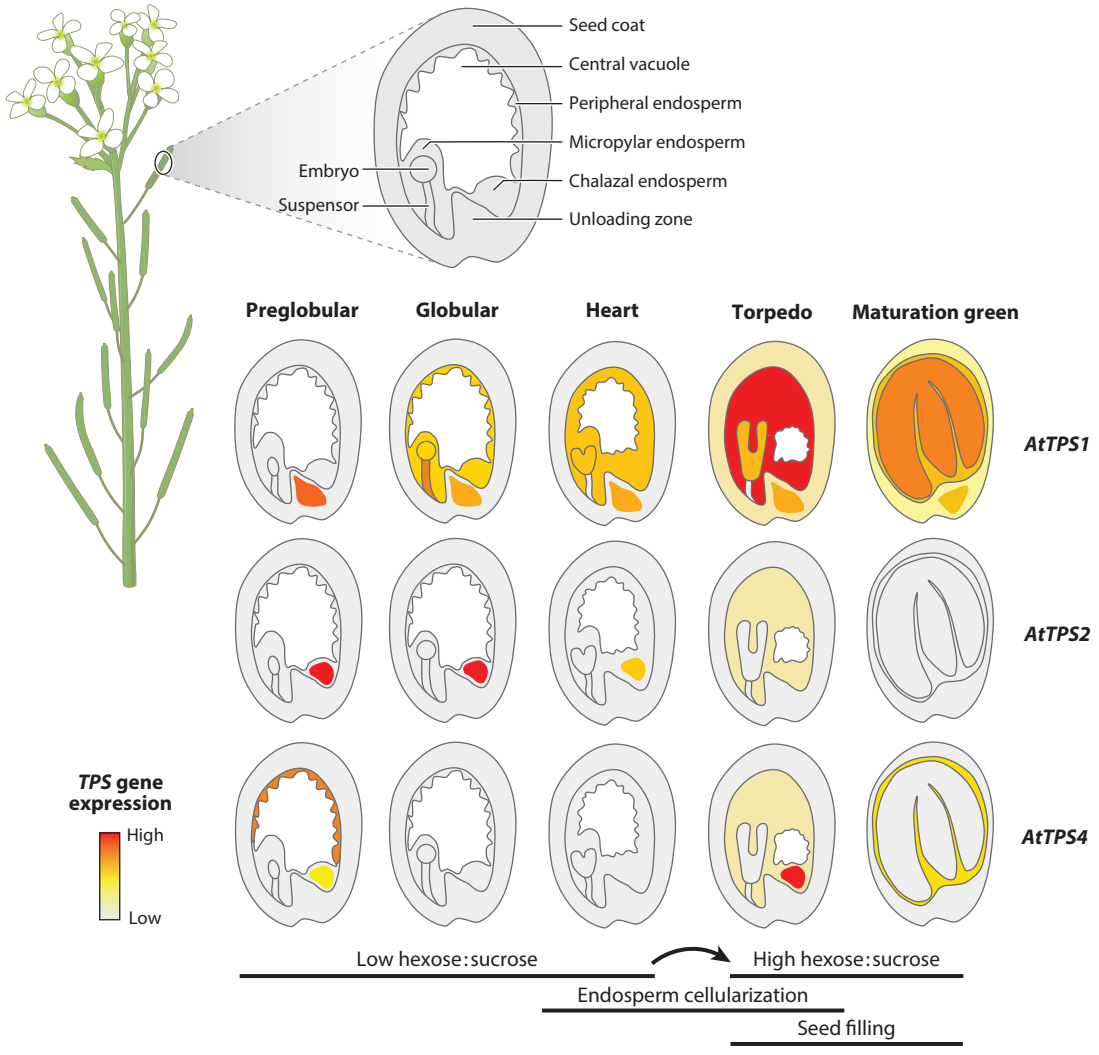

Figure 4

The role of Tre6P in *Arabidopsis* seed development. *AtTPS1* is expressed in the embryo, endosperm, and phloem unloading zone of developing seeds and is essential for embryogenesis, as *tps1* null mutants arrest at the torpedo stage of development (34). Complementation studies showed that the restoration of Tre6P synthesis was necessary and sufficient for the *tps1* mutant to yield viable seeds, demonstrating that embryogenesis is dependent on Tre6P rather than on AtTPS1 per se (38). The functions of Tre6P during embryogenesis are uncertain. The timing of embryo arrest in the *tps1* mutant might indicate a defect in endosperm cellularization, and there is evidence of problems with storage product accumulation during the seed-filling stage (44). Two related class I TPS enzyme genes, *AtTPS2* and *AtTPS4*, are also expressed in developing seeds but are localized to the peripheral and chalazal endosperm (40). Their restricted expression probably accounts for their inability to substitute for the loss of AtTPS1 in *tps1* mutants. Gene expression data are from Reference 57, and the figure is adapted from a display from the Bio-Analytic Resource for Plant Biology (BAR) eGFP browser (**http://bar.utoronto.ca/**) (113). Abbreviation: Tre6P, trehalose 6-phosphate.

The precise functions of Tre6P during embryogenesis are less clear. In maternal tissues, AtTPS1 is located in the vasculature and abscission zones of siliques and in the funiculus and regions of the developing seed where phloem unloading of sucrose and other nutrients (e.g., amino acids) occurs (38) (**Figure 3**). AtTPS1 is also present in filial tissues (38) (**Figures 3** and **4**).

Based on transcript profiling, two other functional members of the class I TPS clade, *AtTPS2* and *AtTPS4*, are also expressed in seeds, but their expression is restricted to the chalazal and peripheral endosperms, whereas *AtTPS1* is expressed in the embryo itself and in the endosperm (38, 40, 57, 105) (**Figure 4**). The highly localized expression of *AtTPS2* and *AtTPS4*, with little or no expression in the embryo and endosperm, probably explains why these two genes cannot compensate for the loss of AtTPS1 in the *tps1* mutant (40).

Axillary bud: the embryonic shoot formed in the leaf axil; often dormant until triggered to grow into a new shoot by internal or external stimuli

Strigolactones: a family of carotenoid-derived phytohormones involved in regulation of shoot branching and root signaling with microbes and parasitic plants

The arrest of *tps1* embryos at the torpedo stage implies a critical function for Tre6P during this stage or in the transition to the final stages of seed development, i.e., storage product accumulation (seed filling) and maturation (dehydration and establishment of seed dormancy). The cellularization of the endosperm begins during the early heart stage of embryo development and is completed during the torpedo stage (15) (**Figure 4**). This process is important for nutrient uptake by the developing seed, coincides with a switch from a high to a low hexose:sucrose ratio in the seed, and is regulated by auxin (12, 56, 73). Mutants that are impaired in endosperm cellularization often fail to produce viable seeds, with embryo arrest in the late heart to early torpedo stages (12, 49), resembling the phenotype of *tps1* embryos. *Arabidopsis* seeds accumulate lipid reserves during the later seed-filling stage of development. This is under the control of the WRINKLED1 transcriptional activator, which is targeted for proteasomal degradation after phosphorylation by SnRK1 (117). The inhibition of SnRK1 by Tre6P therefore promotes lipid accumulation by stabilizing WRINKLED1 (117). In pea (*P. sativum*) seeds, which accumulate mainly starch during the seed-filling stage, Tre6P also promotes storage product accumulation, in this case via the induction of the auxin biosynthesis gene *TRYPTOPHAN AMINOTRANSFERASE RELATED2* (71). The final stage in seed maturation is the establishment of dormancy, which is triggered by the movement of FT, expressed in maternal tissues, to the seed (22, 23, 93). Given the dependence of *FT* expression in leaves on Tre6P, there is potential for Tre6P to also regulate *FT* expression in siliques and thus the onset of seed dormancy. ABA plays a central role in seed dormancy, and seed germination is generally inhibited by ABA. However, this sensitivity is lost in several trehalose pathway mutants (45, 103, 104). It has also been reported that expression of the *ABA INSENSITIVE4* gene, a key player in ABA signaling, is dependent on AtTPS1 (5, 44, 87). These findings strongly implicate Tre6P in ABA signaling and the maintenance of seed dormancy.

4.3. Shoot Branching

Axillary meristems in the leaf axils give rise to axillary buds that usually become dormant after formation and remain quiescent unless triggered to grow out into a new shoot. In many species, the axillary buds remain dormant if the main shoot remains intact, a phenomenon known as apical dominance. Auxin is produced at the shoot tip and transported towards the roots. For many decades, this polar auxin stream was thought to be a key factor in suppressing axillary bud outgrowth, acting via strigolactones and antagonized by cytokinins, which regulate expression of the *BRANCHED1* (*BRC1*) branching repressor (1, 10, 16, 17, 30, 32, 33, 75). However, this phytohormone-based mechanism for repression of axillary bud outgrowth has been questioned by observations that the onset of bud outgrowth after decapitation of pea plants is poorly correlated with auxin levels in the stem (70, 74) but is positively correlated with changes in sucrose supply to the buds (70). Axillary bud outgrowth is also triggered by the exogenous supply of sucrose in pea, *Arabidopsis*, and rose (*Rosa hybrida*) stem explants (11, 13, 37, 70), while dormant buds have a transcript profile that is indicative of carbon starvation (97).

Following decapitation of pea plants, Tre6P levels in axillary buds from near the base of the stem were found to rise within 1–2 h, coinciding with the onset of measurable bud outgrowth (37). Tre6P levels continued to rise for at least 8 h after decapitation, and during this period there were marked changes in the levels of respiratory intermediates and amino acids (37). These findings

suggest that Tre6P might play a role in both the release of bud dormancy when sucrose supply to the buds is increased and the reconfiguration of central metabolism to provide the building blocks needed for growth (37). Although indicative of an important role for Tre6P in the regulation of shoot branching, these correlative results did not prove a causal relationship. To address this, a heterologous TPP was expressed in *Arabidopsis* under the control of the *BRC1* promoter to lower Tre6P levels specifically in axillary buds. The resulting plants initiated rosette branches more slowly than wild-type plants and ended up with fewer branches, indicating that shoot branching is dependent on Tre6P in the buds (36).

AtTPS1 is present in *Arabidopsis* axillary buds (**Figure 3**), showing that they have the potential to synthesize Tre6P themselves in response to changes in sucrose supply (36). It was also found that overexpression of a heterologous TPS in the vasculature promoted shoot branching, whereas overexpression of a heterologous TPP suppressed branching (36), replicating the branching phenotypes of constitutive TPS- or TPP-overexpressing plants (89). This indicated that changes in Tre6P levels in the vasculature outside the axillary buds also influence shoot branching. One possibility is that Tre6P moves systemically in the phloem to axillary buds where, in combination with Tre6P made in the buds themselves, it promotes bud outgrowth. Expression of several *SWEET* genes, encoding sucrose efflux carriers, was upregulated in the plants with overexpression of TPS in the vasculature, potentially enhancing the loading of sucrose into the phloem in source leaves and the unloading of sucrose in sink organs, including the axillary buds (36) (**Figure 2**). It is unknown whether the upregulation of *SWEET* expression was associated with increased wall ingrowths in phloem parenchyma transfer cells, whose development is regulated by sucrose, independently of Tre6P (110). The enhanced branching phenotypes in plants with TPS overexpression in the vasculature were shown to be dependent on FT, which can move to axillary buds, as well as the SAM, to control the floral transition in the axillary bud meristem and growth of the bud into a new shoot (76, 99). These findings reveal the potential for Tre6P to generate long-distance signals in plants, acting indirectly by modifying the transport and allocation of sucrose and by modulating the expression of FT. It is also possible that Tre6P itself is a long-distance signal, moving within the phloem from source to sink organs to regulate metabolic and developmental processes in the latter. If Tre6P were shown to move over long distances and have an impact on distal parts of the plant, this would qualify Tre6P to be counted as a new type of phytohormone.

SUMMARY POINTS

1. Trehalose metabolism is essential for normal plant growth and development, and trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is a signal and regulator of sucrose levels in plants.
2. In *Arabidopsis* leaves, Tre6P is produced in the phloem companion cell-sieve element complex, phloem parenchyma, and stomatal guard cells.
3. Tre6P made in the phloem parenchyma of the bundle sheath regulates photosynthetic sucrose production and remobilization of transitory starch degradation in mesophyll cells to balance sucrose supply with demand from growing sink organs.
4. As a signal of sucrose availability, Tre6P influences developmental transitions that will increase demand for sucrose in the future, such as flowering and shoot branching.
5. Tre6P promotes flowering by regulating expression of *FT* in phloem companion cells in the leaf vasculature and via interaction with components of the age-dependent pathway in the shoot apical meristem.

6. Tre6P is essential for embryogenesis, influencing seed filling and dormancy, but its precise functions during seed development are poorly characterized.
7. Outgrowth of axillary buds into new shoots is dependent on Tre6P, which is involved in the breaking of bud dormancy by changes in sucrose supply and *FT* expression and the subsequent reconfiguration of bud metabolism for growth.
8. In growing sink organs, Tre6P regulates the utilization of sucrose for growth and the accumulation of storage reserves, in part via complex interactions with SnRK1.

FUTURE ISSUES

1. Our knowledge of the regulation of plant trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) enzymes is fragmentary, limiting our understanding of how Tre6P responds to fluctuations in sucrose levels.
2. The recognition that some TPP enzymes have noncatalytic moonlighting functions in developmental regulation was a major breakthrough, but further study is needed to understand those functions and why plants have such large and diverse TPP families.
3. The class II TPS proteins in plants, which appear to lack TPS and TPP activities, deserve greater attention to elucidate their functions and determine whether they contribute to Tre6P metabolism and signaling and, if so, how.
4. The use of cell- and/or tissue-specific promoters to manipulate Tre6P is a promising approach for dissecting the functions of Tre6P, and inducible promoters provide temporal resolution to distinguish between primary and secondary effects of changes in Tre6P levels.
5. The development of sensitive and specific in vivo reporters of Tre6P would provide a quantum leap forward in our ability to investigate Tre6P signaling.
6. Heterografting experiments to test whether Tre6P is a long-distance signal in plants should be a high priority.
7. Further in vitro reconstitution experiments are needed to understand the complex interactions of Tre6P with SnRK1, along with ligand-binding assays and screening of protein libraries to identify other Tre6P targets.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by the Australian Research Council [postdoctoral fellowship to F.F. associated with the Georgina Sweet Laureate Fellowship (FL180100139) awarded to Professor Christine Beveridge] and the Max Planck Society (J.E.L.).

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