Metabolism control over growth: a case for trehalose-6-phosphate in plants

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Abstract

How plants relate their requirements for energy with the reducing power necessary to fuel growth is not understood. The activated glucose forms and NADPH are key precursors in pathways yielding, respectively, energy and reducing power for anabolic metabolism. Moreover, they are substrates or allosteric regulators of trehalose-phosphate synthase (TPS1) in fungi and probably also in plants. TPS1 synthesizes the signalling metabolite trehalose-6-phosphate (T6P) and, therefore, has the potential to relate reducing power with energy metabolism to fuel growth. A working model is discussed where trehalose-6-phosphate (T6P) inhibition of SnRK1 is part of a growth-regulating loop in young and metabolically active heterotrophic plant tissues. SnRK1 is the Snf1 Related Kinase 1 and the plant homologue of the AMP-dependent protein kinase of animals, a central energy gauge. T6P accumulation in response to high sucrose levels in a cell inhibits SnRK1 activity, thus promoting anabolic processes and growth. When T6P levels drop due to low glucose-6-phosphate, uridine-diphosphoglucose, and altered NADPH or due to restricted TPS1 activity, active SnRK1 promotes catabolic processes required to respond to energy and carbon deprivation. The model explains why too little or too much T6P has been found to be growth inhibitory: Arabidopsis thaliana embryos and seedlings without TPS1 are growth arrested and Arabidopsis seedlings accumulating T6P on a trehalose medium are growth arrested. Finally, the insight gained with respect to the possible role of T6P metabolism, where it is known to alter developmental and environmental responses of plants, is discussed.

Key words: Arabidopsis, bZIP11, carbon allocation, carbon signalling, development, growth, Single Nucleotide Polymorphism SnRK1, trehalose-6-phosphate, trehalose-6-phosphate synthase.

Plant trehalose-6-phosphate synthesis starts from two activated glucose metabolites central to metabolism

Trehalose is the α-1,1-linked glucose disaccharide and, consequently, a non-reducing stable sugar widely found in living cells of bacteria, archaea, fungi, invertebrates, and plants (Avonce et al., 2006). The synthesis of trehalose in plants is accomplished via the phosphorylated intermediate, trehalose-6-phosphate (T6P) (Fig. 1A). Substrates for the synthesis of T6P are the activated forms of Glc uridine-diphosphoglucose (UDPG) and glucose-6-phosphate (G6P). Both are central to plant metabolism (Masakapalli et al., 2010). UDPG is known as the starting point for polysaccharide biosyntheses including cell wall cellulose and callose. G6P is the starting point for energy metabolism. G6P is also used to generate NADPH for reductive biosyntheses, particularly in heterotrophic tissues.

T6P synthase (TPS) activity was demonstrated for Arabidopsis TPS1. TPS1 activity is essential because TPS1 deletion mutants were found to be embryo lethal (Blazquez et al., 1998; Eastmond et al., 2002). Yet there are another 10 homologues to TPS1 in Arabidopsis whose function remains
enigmatic (Leyman et al., 2001; Avonce et al., 2006; Ramon et al., 2009; Vandesteene et al., 2010). T6P phosphatase (TPP) activity was demonstrated for all 10 TPP homologues in Arabidopsis (Vandesteene, 2009; Vogel et al., 1998). Alternatively, G6P can be converted into fructose-6-phosphate and, together with UDPG, be used for the synthesis of sucrose-6-phosphate (S6P) and then sucrose (Suc); Suc synthesis is continuous in plant cells and S6P levels, unlike T6P levels, can vary a lot with relatively minor effects on plant growth (Chen et al., 2005). The abundance of TPS/TPP domain proteins contrasts with the single trehalase protein identified thus far in Arabidopsis; the trehalase enzyme activity hydrolyses trehalose (Tre) into two Glc molecules (Aeschbacher et al., 1999; Muller et al., 2001).

We have yet to understand the function of the plant TPS homologues, as most of the TPS/TPP domain proteins lack the ability to complement TPS- or TPP-deficient yeast strains. One possibility is that some are pseudo-genes, as it is been proposed for TPS3 (Vandesteene, 2010) or they...
have acquired new functions. An indirect way to assess the functionality is to analyse the selective pressure at the amino acid level for each TPS and TPP domain (Avonce, 2006). To evaluate the importance of individual proteins of T6P metabolism for plants in the wild, their natural variation was analysed in the 81 accessions of Arabidopsis thus far sequenced (http://1001genomes.org/data/MPI/MPICao2010/releases/2010_03_25/; Cao et al., 2011). The natural variation analysis was restricted to Single Nucleotide Polymorphisms (SNP) compared with the reference sequence of accession Col.0 for each gene associated with trehalose metabolism. The selective pressure is considered for each gene by calculating the ratio of SNP that changes the amino acid sequence (non-synonymous SNP) with SNP that do not (synonymous SNP). The analysis revealed that some of the TPS and TPP proteins are extremely conserved (Fig. 1B). TPS6, 5, and 7, in particular, have a very low ratio of non-synonymous to synonymous SNP, much lower than the essential TPS1. In addition, TPS1, G, and A have a very low ratio of non-synonymous to synonymous SNPs. Over all the 81 accessions sequenced, only TPS2 and TPS3 were found with a premature stop deleting either the TPS and TPP or just the TPP domain, suggesting TPS2 and T3 are likely to be dispensable pseudo-genes as suggested earlier (Lunn, 2007). There is also a surprising amount of natural variation in the enzymatic domain of TRE1, the only annotated trehalase in Arabidopsis.

**Lessons learnt from fungi on possible roles of the TPS enzyme in the regulation of growth and development**

From the budding yeast Saccharomyces cerevisiae it is known that T6P, as well as the Tps1 enzyme complex, inhibits the entry of glucose into metabolism; T6P inhibits the hexokinase activity of Hxk2 (Blazquez et al., 1993; Blazquez and Gancedo, 1994; Bell et al., 1998; Noubhani et al., 2000; De Silva-Udawatta and Cannon, 2001; Bonini et al., 2003). The Tps1 enzyme complex and T6P, therefore, relate entrance of glucose into metabolism with levels of the T6P substrates G6P and UDPG as well as with other factors for growth that have yet to be identified but probably include NADPH (see below).

Yeast without Tps1 is unable to grow on Glc; the cells are depleted in ATP and Pi whilst phosphorylated hexoses including G6P accumulate. Deletion of Hxk2 suppresses the growth arrest on Glc medium and the sporulation defect of yeast without Tps1.

Hxk2 has another function besides permitting hexoses to enter metabolism by phosphorylation: the enzyme also serves as a factor that mediates glucose repression, a term coined to describe transcriptional changes for metabolism of less suited substrates once glucose is no longer available. In the nucleus, Hxk2 occurs in a complex with Sucrose-non fermenting-1 protein kinase (Snf1) (Ahuatzi et al., 2007; Pelaez et al., 2010). Snf1 protein kinase is the yeast homologue of the central animal AMP-activated protein kinase that relates levels of AMP (marking low energy status when it accumulates) with cellular responses to energy deprivation. Snf1 phosphorylation of enzymes in the cytosol accounts for short-term adjustments to low energy levels. Snf1 phosphorylation of transcriptional machinery components and histones in the nucleus is thought to account for longer-term adjustments to low energy levels (De Virgilio, 2011). When Glc is available, Hxk2 inhibits Snf1 from phosphorylating the Mig1 repressor; when not phosphorylated, Mig1 inhibits transcription of the invertase Suc2 as well as some other targets of glucose repression. The Hxk2/Snf1 complex relates available substrate and energy with glucose repression. It has yet to be discovered, however, how Tps1 and T6P interfere with the functions of the Hxk2/Snf1 complex.

The Tps1 enzyme can be found in a large complex with the Tps2, an active TPP, as well as Tps3 and Tsl1 (De Virgilio et al., 1993; Bell et al., 1998). Tps3 and Tsl1 are homologues of Tps1 but appear catalytically inactive. They are not essential for growth on Glc but seem to be required for an optimum function of Tps1 activity and, therefore, are likely to have a regulatory function.

To conclude, information from the budding yeast reveals that T6P/Tps1 controls Hxk2 enzyme activity; Hxk2 has another function, however, and also occurs in the nucleus in a complex with Snf1 kinase. The Hxk2/Snf1 complex mediates the glucose repression of genes required for the metabolism of carbohydrate other than Glc. In addition, Hxk2/Snf1 complex activity is important during energy stress responses. Yeast has two more Tps1 homologues that complex with Tps1/Tps2, these Tps3 and Tsl1 proteins have regulatory but not catalytic functions that are poorly understood (Fig. 2A).

The rice blast fungus, Magnaporthe oryzae, is less specialized in its carbon nutrient supplies compared with S. cerevisiae. Consequently, T6P does not inhibit hexokinase activity in this organism (Wilson et al., 2007, 2010).

Instead, work in M. oryzae has shed some light on other interesting functions of the Tps1 enzyme. A clear function of the Tps1 enzyme as a sensor for G6P was shown; this function is independent of the T6P synthase activity. Mutants with catalytically inactive Tps1 which retained an intact G6P binding site were able to restore the infectivity of tps1Δ mutants. The G6P sensing by Tps1, therefore, is important for a development of the fungus in its plant host.

In addition, M. oryzae without Tps1 have low G6P-dehydrogenase (G6PDH) activity. G6PDH is the first enzyme of the oxidative phase of the pentose phosphate pathway (OPP) that generates NADPH. G6PDH is also the rate-limiting regulated enzyme of the OPP. Tps1 is therefore required for the up-regulation of NADPH biosynthesis in the OPP. NADPH is generally required for reductive biosyntheses in most cells and, more specifically, for the reduction of NO3 to NO2 by M. oryzae nitrate reductase. Consistently, M. oryzae Tps1 mutants were unable to thrive on medium with NO3 as substrate, probably because of a lack of the NADPH reducing cofactor to assimilate NO3 into NO2 by nitrate reductase.
finite amount of the NADP cofactor, G6PDH control regulates NADP-dependent processes. For instance, when NADPH levels are low, high NADP allosterically binds Nmr1 factors which then no longer inhibit transcription factors commanding the expression of G6PDH of genes involved in processes requiring NADPH and in virulence and secondary metabolism.

Tps1 from *M. oryzae* can fully complement the phenotypes of *tps1Δ* in *S. cerevisiae*. T6P synthesized by the *M. oryzae* Tps1 inhibits Hxk2 from yeast; in addition Tps1 activation of G6PDH after sensing G6P further would possibly reduce G6P accumulation and, therefore, apparent flux through hexokinase.

Together the data suggests that fungal Tps1 is central in regulating the pathways generating both energy and reducing power for biosynthetic processes. Direct or indirect inhibition of hexokinase activity (or/and possibly signalling) and flux through glycolysis will ultimately control the amount of ATP generated energetically to couple reactions in growth processes. Activation of G6PDH as a function of available NADPH controls the amount of reducing power required for reductive biosyntheses that allow lipid biosyntheses and, therefore, membrane extension, for example. Tps1, therefore, is central in controlling the provision of metabolite substrates, carbon, energy, and redox power, for growth (Fig. 2C). Because of its control over reducing power in the form of NADPH, Tps1 is also important for the assimilation of nitrate by nitrate reductase when carbon to nitrogen ratios are high and so relates carbon with nitrogen availability.

**T6P is required for carbon utilization in the plant Arabidopsis**

In *Arabidopsis*, just like in *M. oryzae*, hexokinase activity is not inhibited by T6P (Eastmond *et al.*, 2002; Baud and Graham, 2006; Gomez *et al.*, 2006). Deletion of TPS1 in plants, however, is embryo lethal, suggesting an essential function for this enzyme. Embryos of the TPS1 knockout mutant *tps1* are much delayed in their development and eventually stop any further development at torpedo stage. The *tps1* embryos are supersensitive to exogenously supplied sugars. Similarly, seedlings with reduced levels of T6P steady-state are supersensitive to exogenously supplied sugar (Schluepmann *et al.*, 2003). By contrast, seedlings with increased levels of T6P steady-state thrive on external sugar and have increased growth when compared with the wild type. Therefore, it was proposed that T6P is required for carbon utilization.

T6P in plants was shown to inhibit the Snf1 Related Kinase 1 (SnRK1) activity (Zhang *et al.*, 2009; Paul *et al.*, 2010). SnRK1 kinase is the plant homologue of animal AMP-activated protein kinase and yeast Snf1 and has retained the same heterotrimeric complex structure. The SnRK1 complex contains α, β, and γ subunits (Halford and Hey, 2009). The α subunits are catalytic and encoded by the two homologous genes KIN10 and KIN11. The γ subunit...
encoded by SNF4 probably binds AMP (Lumbreras et al., 2001). KIN10 over-expression in Arabidopsis seedlings causes dramatic reprogramming of gene expression after 6 h (Baena-González et al., 2007). The expression profile obtained was similar to that of seedlings with carbon nutrient or energy stresses, either due to extended darkness, inhibition of photosynthesis or submergence. Increased SnRK1 therefore mediates these low-energy stress responses. Increased SnRK1 causes expression re-programming promoting catabolic processes, in particular, the remobilization of protein in which Asparagine Synthase 1 (ASN1) is involved (Baena-Gonzalez and Sheen, 2008). Moreover, the gene-expression profile obtained after increased SnRK1 was opposite to that obtained when feeding carbon in the form of gluc, sucrose (Suc) or CO₂, suggesting that in yeast but T6P amounts to a conserved effect linking energy status with growth by way of Snf1. The ratio of NADPH/NADP⁺ within 15 min after incubation with Tre is not significantly altered whilst the enzyme is very much redox-activated already (Kolbe et al., 2005). Feeding Tre for 3 h, however, significantly reduced the ratio of NADPH/NADP⁺ as well as the levels of G6P. This reduction is not seen when feeding Suc. It is possible that NADPH/NADP⁺ measured after 15 min was from a large pool of NADPH, which may not reflect the rapid changes that may occur specifically in the chloroplast. It is therefore still possible that T6P accumulation may cause NADPH depletion. (Alternatively, the large flux of G6P into starch may cause a reduction of substrate for the OPP pathway and then depletion of NADPH. Yet this is improbable because the experiment was done in leaf tissue in the light which would regenerate NADPH from NADP.)

Too much T6P in the absence of exogenously supplied carbon stops growth

Feeding Tre for longer periods of time is surprisingly toxic to some of the plants tested. As early as 1981, Cuscuta reflexa (dodder) shoots were fed Tre and shown to be growth-inhibited as a consequence (Veluthambi et al., 1981, 1982a, b). The inhibition of growth was located in the extension zones of the shoot tip, even though Tre was transported and accumulated throughout the shoots. The inhibition correlated with a reduced amount of carbon incorporation from radiolabelled Glc into soluble and
insoluble fractions at the shoot tip. Growth inhibition was suppressed when both Tre and Suc were fed simultaneously.

*Arabidopsis* seedlings, like *C. reflexa* plants have low levels of trehalase activity in their tissues (Wingerl et al., 2000; Muller et al., 2001). *Arabidopsis* seedlings are similarly growth-inhibited when grown on media with Tre and the effect is suppressed when feeding Suc or any metabolizable sugar simultaneously with Tre.

Growth inhibition of *Arabidopsis* seedlings is particularly interesting because it is associated with a large accumulation of starch in cotyledons. By contrast, root tips that fail to grow do not show the typical accumulation of starch in the columnella cells (Wingerl et al., 2000; Schluepmann et al., 2004). Tre feeding thus disturbs carbon allocation with all the carbon remaining in the source cotyledons and apparent carbon starvation at the root and shoot apices.

The growth arrest on Tre was shown to be caused by a 10-fold accumulation of T6P compared with osmoticum control (Schluepmann et al., 2004). The causal relationship was supported mainly by the fact that seedlings expressing *E. coli* T6P-hydrolase grew on Tre. The T6P hydrolase splits T6P into gluc and G6P and the free energy ΔG° of this reaction is not influenced by Tre levels in the cells. This is unlike the ΔG° of the TPP reaction which will be influenced by the very high (>100 000:1 ratio) of Tre to T6P.

It was hypothesized at first that T6P accumulation may cause starch accumulation in the cotyledons due to its effect on AGPase redox, thus sequestering all carbon to the source organs. But mutants in plastidic phospho-glucomutase 1, pgm1, do not accumulate any starch and are similarly growth-inhibited as the wild type on Tre. It was, therefore, concluded that T6P’s effect on AGPase and carbon sequestration into starch were not the cause for growth inhibition.

Instead, T6P inhibition of SnRK1 activity was shown to cause growth inhibition on Tre because seedlings over-expressing KIN10 grew on Tre (Delatte et al., 2011). Moreover, a subset of SnKR1 targets was shown to be repressed on Tre. The targets included typical stress-associated proteins such as SEN1 (Senescence associated protein 1) and ASN1 (Baena-Gonzalez and Sheen, 2008; Delatte et al., 2011). These targets were common to SnKR1 and bZIP11, the S1-class basic leucine ZIP 11 transcription factor identified as one of the potential transcription factors mediating the transcriptional responses controlled by SnRK1 (Baena-Gonzalez et al., 2007). Consistently, seedlings over-expressing bZIP11 also grew on Tre (Delatte et al., 2011).

When feeding trehalose (Tre) at 100 mM which slows T6P dephosphorylation and causes high T6P, irrespective of the amount of carbon substrate for growth; growth ceases because the cells are unable to sense the depletion of hexose substrates and to respond so as to induce processes required to replenish substrates. Feeding Tre failed to cause uORF2-mediated sucrose repression of bZIP11 translation, and Tre growth inhibition could be suppressed by over-expression of the putative target of SnRK1, bZIP11. Dashed arrows are used when firm experimental evidence for the link is lacking.
et al., 2011). Over-expressors of bZIP11 grew in spite of elevated T6P, suggesting that they were insensitive to low SnRK1 activity.

Overall, results showed that SnRK1 is required for growth at the heterotrophic apices in seedlings of Arabidopsis. Consistently, SnRK1 antisense barley and potato plants showed that SnRK1 activity is required in heterotrophic tissues for growth and starch synthesis.

Together, data suggest that seedlings on Tre stop growing because high Tre causes T6P accumulation that, in turn, inhibits SnRK1 activity. The conclusion may seem paradoxical because both too little T6P, in the tps1 mutant, and too much T6P, when feeding Tre, are growth inhibitory.

The mechanism by which T6P/SnRK1 exerts metabolic control over growth in plants is far from understood, but given the few known elements presented above, a working model is proposed. Such a model would explain the paradox of growth regulation by T6P if T6P/SnRK1 were part of a regulatory loop (Fig. 3B). In heterotrophic tissues, T6P increases when Suc is available. The T6P increase inhibits SnRK1 activity such that cells are allowed to go about anabolic processes required for growth. When Suc levels or carbon availability drops, then T6P levels drop, unleashing SnRK1 activity. SnRK1 activity is known to be activated during carbon and energy stress responses. SnRK1, therefore, is required to signal low energy or carbon that are sensed and then mediate processes required to make carbon available in sink cells for growth. SnRK1 activity is required until carbon becomes available again in the heterotrophic tissues. Suc is then synthesized and T6P levels rise again sufficiently to inhibit SnRK1 activity.

During Tre feeding, T6P accumulates, irrespective of the available carbon, and SnRK1 inactivation causes induction of cellular process for anabolism: the growth response is uncoupled from energy and carbon availability. This would explain the relative susceptibility to Tre of growing sink tissues over metabolically less active tissues.

Conflicting results may require elaborating the working model of the T6P/SnRK1 regulatory loop

The intermediary factor, factor I, and the heterogeneity of SnRK1 may be the reasons for the controversial results observed and for the differing interpretations with respect to the SnRK1 effect on growth. Firstly, T6P inhibition of SnRK1 requires factor I which is not present in mature source leaves (Zhang et al., 2009). Factor I is likely to be a protein: it can be inactivated by boiling; it is also likely to be soluble since soluble extracts from seedlings can be added to render SnRK1 from mature leaf susceptible to T6P inhibition. The latter is an important observation because it also shows that factor I is dominant in in vitro enzyme assays; it therefore may be that, even in young seedlings, not all of the tissues contain factor I.

Heterogeneity of the SnRK1 complex within the differing tissues of the seedlings has been demonstrated recently using KIN10GFP and SNF4YFP protein fusions: KIN10GFP was functional in vitro and was found at the plasma membrane in all tissues along with SNF4 (Bitrian et al., 2011). In cells of young tissues at both apices of Arabidopsis, however, KIN10GFP is accumulating in regions surrounding the nuclei whilst SNF4YFP is found within the nuclei. This is not the case in cells of the cotyledons or hypocotyls. It could, therefore, be that KIN10 regulation differs in young growing tissues from tissues of the cotyledons and hypocotyls. Factor I, for example, may only be present in young tissues of seedlings but not in cotyledons, restricting T6P inhibition of SnRK1 to specific tissues.

SnRK1 was shown to be required for potato AGPase redox activation after just 1 h of Tre feeding (Kolbe et al., 2005). Incubation of isolated chloroplast preparations with T6P (and ATP) led to AGPase redox activation and suggested that T6P may be transported into the plastid where it may act independently of SnRK1 to cause redox activation. SnRK1 activity may, therefore, be required before T6P enters the plastid. SnRK1 may be required to provide the substrate G6P for NADPH production in the plastids. The absence of AGPase redox activation in antisense SnRK1 potato slices, however, occurred when the NADPH/NAD+ ratio as well as G6P levels remained unchanged compared with the wild type. Without information on the subcellular distribution of these compounds, it is difficult to determine whether T6P is upstream or downstream of SnRK1 in the regulation of AGPase redox regulation. Further research is needed here, but the genetic evidence in seedlings over-expressing KIN10 suggests that T6P is upstream of SnRK1 when considering growth processes in heterotrophic seedling tissues of Arabidopsis (Delatte et al., 2011).

The SnRK1 requirement for redox activation was not yet shown for leaves and it has yet to be seen whether plants with low SnRK1 activity show AGPase redox activation in their leaves upon Tre feeding (Kolbe et al., 2005). Nevertheless, WT seedlings show massive starch accumulation in cotyledons as a consequence of Tre feeding, indicating that redox-activation of AGPase functions in cotyledons (Wingler et al., 2000). SnRK1 may, therefore, not be required in cells when energy and carbon for starch synthesis are available in chloroplasts from photosynthesis. Wingler et al. (2000) have shown that starch accumulation is derived from carbon fixed by photosynthesis because starch does not accumulate in seedlings on Tre grown in the dark.

T6P is a charged metabolite; it is therefore unlikely to be exported to other cells and it is expected to function cell autonomously. Growth inhibition in seedlings of Arabidopsis only acts once the seedling has expanded, possibly when heterotrophic tissues have exhausted their lipid stores (Flugge et al., 2011). Tre inhibition of growth becomes visible once seedling growth occurs at the apices which rely on an external supply of carbon (and nitrogen) (Wingler et al., 2000). T6P inhibition of SnRK1 could therefore be critical for carbon loading of the phloem, carbon supply to the apices from the phloem, or the presence of carbon in the
cells of the apices. SnRK1 was shown to act cell autonomously during barley pollen development: the SnRK1 antisense pollen was unable to undergo full development and unable to accumulate starch whilst the WT segregants from heterozygous SnRK1 antisense plants were unscathed (Zhang et al., 2001). If applicable to the situation in growing apices of Arabidopsis, the result would suggest that SnRK1 activity is required in cells of the growing tissues. This is consistent with high levels of SnRK1 found during the early phases of wheat grain development (Martinez-Barajas et al., 2011). The fact that these early phases of wheat grain development are also characterized by as yet unseen levels of natural T6P accumulation as well was puzzling at first, but is consistent with the proposed regulatory loop. When Suc or C/N ratio are high, high T6P with consequent rapid and full inactivation of SnRK1 would signal that carbon may be used for energy and reducing power for growth and, therefore, increase sink strength of these young tissues in developing wheat grains. By contrast, high SnRK1 activity is required for these metabolically hyperactive cells rapidly to transduce the signal from low levels of energy or carbon to check their anabolic processes against available substrate (Lunn et al., 2006; Martinez-Barajas et al., 2011; Delatte et al., 2011; H Schluepmann, unpublished data). Due to the high growth rates in young tissues high SnRK1 and T6P steady-states can therefore be expected.

That SnRK1 and T6P are needed in cells in growing heterotrophic tissues does not exclude that they are not also required in the phloem companion cells, since these cells are heterotrophic and rely on Suc (Flugge et al., 2011). These cells are also particularly metabolically active. The high translation rates of KIN10 and bZIP11 in the phloem companion cells, where the sucrose transporter AtSUC2 is expressed, suggests this (Fig. 4A) (Mustroph et al., 2009; Mustroph and Bailey-Serres, 2010). Translation in the specific cell files was evaluated in polysomal mRNA fractions that immunoprecipitated with the ribosomal protein RPL18 using plant lines expressing epitope tagged RPL18 behind specific promoters, for example, the AtSUC2 promoter (Mustroph et al., 2009). Accumulation of soluble sugars over all in seedlings on Tre is remarkable considering that an external supply of sugars is required to suppress growth inhibition (Delatte et al., 2011). It is consistent with Tre feeding disturbing carbon allocation and thus roles of SnRK1 in phloem companion cells that mediate transport. Veluthambi et al. (1982b) already showed that Tre feeding decreases Suc transport to the shoot apex. Further research is needed to reveal where soluble sugars accumulate in seedlings on Tre, but it is possible that, with inactive phloem transport, the sugar synthesized may accumulate in source organs along with starch.

In seedlings grown on Tre that accumulate high levels of soluble sugars, uORF2 surprisingly did not suppress bZIP11 translation (Wiese et al., 2005; Delatte et al., 2011). uORF2 is the upstream open reading frame 2 found in the 3′ leader of the bZIP11 mRNA. uORF2 is required for translational repression of bZIP11 when Suc accumulates to high levels. Lacking suppression of translation in seedlings on Tre that accumulate sugars raises the question of where the soluble sugars accumulate: they may be shunted into the plastid or vacuole and hence be unavailable to metabolism or signalling in the cytosol. Alternatively, the sugars are not sensed because the signal is not transduced in the absence of SnRK1 activity; SnRK1 may be required for uORF2 repression of translation when Suc accumulates. Transduction after sensing may also be required for soluble sugar export from source tissues and may explain the accumulation of soluble sugars in source tissues.

Surprisingly, over-expression of KIN10 or bZIP11 did not suppress the accumulation of soluble sugars despite suppressing growth inhibition (Delatte et al., 2011; Ma
et al., 2011). Quite the opposite in fact: soluble sugar accumulation was much increased. This is consistent with the proposed roles of KIN10 and bZIP11 in catabolism mobilizing, for example, proteins to provide substrate to maintain growth under stress (Baena-Gonzalez and Sheen, 2008). In the case of bZIP11 over-expression, increased soluble Suc is also consistent with the high T6P levels measured in seedlings even under control growth conditions. Linking tissue-specific promoters such as the promoters from the genes STM, WOX or SUC2 (Mustroph et al., 2009) to KIN10 or bZIP11 would indicate where T6P inhibition of SnRK1 interferes with growth on Tre.

It is not known in which tissue T6P accumulation is critical for the arrest of growing tissues in seedlings. A preliminary deduction could be that T6P inhibition of SnRK1 promotes anabolic processes in every cell where T6P accumulates as a result of Tre feeding. The growth processes are not coupled to available energy or carbon because T6P no longer reflects levels of Suc or its biosynthetic precursors in the cells. In cells with carbon from photosynthesis, the carbon is, furthermore, used for anabolic processes leading to sequestration of soluble sugars and starch. In heterotrophic cells, however, carbon resources are depleted entirely and growth ceases. More SnRK1 and bZIP11 counteract by promoting catabolic processes that will render carbon available to heterotrophic tissues, such as the phloem companion cells or cells of the growing apices. bZIP11 causes accumulation of soluble sugars probably because it promotes the ASN1-dependent mobilization of protein and inhibits TCA. That Tre feeding interferes with sugar availability in growing tissues is not new given the results from sugar feeding experiments carried out by Veluthambi et al. (1982b) and Wingler et al. (2000). The recent data reveal that the inability to synthesize sugars in growing tissues on Tre may be because tissues are unable to sense a lack of soluble sugars when forcible T6P accumulation inhibits SnRK1 and so triggers anabolic processes irrespective of the carbon available to fuel growth.

In conclusion, the major conflict between the regulatory loop model and experimental evidence is that SnRK1 seems active both upstream and downstream of T6P in the case of AGPase redox activation in heterotrophic tissues (Kolbe et al., 2005; Michalska et al., 2009). Upon closer inspection, however, it has yet to be shown that T6P enters plastids and whether SnRK1 promotes transport of substrate such as G6P into plastids of heterotrophic tissues. But perhaps there is some truth to SnRK1 acting upstream of T6P because SnRK1 was proposed to phosphorylate TPS homologues such as TPS5 and TPS8 which probably interact with TPS1 and so affect the synthesis of T6P as a function of Suc (Glinski and Weckwerth, 2005; Harthill et al., 2006). Heterogeneity was expected in the T6P inhibition of SnRK1 between heterotrophic tissues and photoautotrophic tissues (Bitrian et al., 2011). The regulatory loop model is far from complete. Comparison with the fungal system shows that some components of the regulatory loop are conserved whilst the large number of plant TPS seemingly interacting with TPS1 predict a more differentiated response of TPS1/T6P signalling in line with increased organ differentiation in plants. Future biochemical work should test the properties of the TPS1/TPS protein complexes from plants with those known in fungi as these could also be G6P and NADPH sensors.

Towards an improved understanding of the role of trehalose metabolism in developmental processes and in responses to environmental changes

It has been established that the SnRK1/T6P regulatory loop mediates metabolite control over growth. This allows an understanding of the dramatic developmental effects observed when T6P accumulates forcibly, but also when T6P cannot be synthesized because the TPS1 enzyme and T6P is lacking in tps1 torpedo stage embryos (Eastmond et al., 2002; Schluepmann et al., 2003). It is possible that SnRK1 activity is unchecked in these embryos, phosphorylating, and thus inactivating, metabolic enzymes under its control. Admittedly, KIN11 but not KIN10 is expressed during embryonic development (Bitrian et al., 2011) and, therefore, it has yet to be confirmed that SnRK1 with KIN11 as the catalytic subunit from Arabidopsis embryos is also inhibited by T6P. Nevertheless, the relative inactivity of enzymes in the torpedo stage tps1 embryos has been reported earlier (Baud and Graham, 2006). Remarkably, G6PHD was inactive in tps1 mutant embryos compared with a high activity in the WT at 12 DAF, suggesting that TPS1 may be required to activate G6PDH in plants as it is required in M. oryzae (Fig. 4B). AGPase was also inactive and starch levels reduced (Baud and Graham, 2006; Gomez et al., 2006). Overall the enzyme activities of tps1 embryos are consistent with a quiescent state imposed by high SnRK1 activity; the role of Snf1 in yeast quiescence is well documented (De Virgilio, 2011).

Very little is known about the single domain plant TPP enzymes. RAMOAS3 (RA3) is required for development in maize inflorescences and was shown to be an active TPP from a grass-specific TPP clade (Satoh-Nagasawa et al., 2006). We fail to understand the mechanism of its action, but, in principle, restricted removal of T6P from a defined subset of cells in a meristem may be an avenue to impose growth restriction on specific cells by activating SnRK1 and so control the shape of developing organs. The tissue-specific expression and translation of some members in the TPP and TPS families is remarkable (Fig. 4B) (Satoh-Nagasawa et al., 2006; Mustroph et al., 2009; Ramon et al., 2009).

It has also become obvious that expression of a number of the TPP and TPS enzymes (except TPS1) is exquisitely responsive to environmental challenges. For example, low oxygen, meaning low energy associated with a requirement to switch to fermentation, seems to activate translation of TPPA and TPS8 in roots of Arabidopsis (Fig. 4C) (Mustroph et al., 2009). T6P inhibition of SnRK1 is expected to be abolished when cells are exposed to energy or carbon.
depletion; abolishing T6P inhibition could be done by sequestration or dephosphorylation of T6P and would explain the role of TPP. It is possible that the plant TPS modulate the activity or stability of the TPS1 complex and thus its function in response to environmental stimuli, as proposed earlier by Vandesteene et al. (2010). A complex set of interactions between the differing TPS has indeed been shown for rice; OsTPS1 specifically interacts with OsTPS8 and OsTPS3 to form a complex of about 360 kDa, but it is not yet known whether TPS1 in the complex is active with respect to T6P synthesis (Zang et al., 2011). The N-terminal part of the TPS including the TPS domain was shown to be sufficient to mediate the interactions between OsTPS1 and OsTPS5 and OsTPS8. When considering the phylogenetic relations of the TPS domains, OsTPS5 and OsTPS8 do not, however, have a close homologue in Arabidopsis. Nevertheless, it is envisaged that the multiple sensing of G6P, UDPG, NADPH, and AMP by TPS1 and SnRK1 complexes may require further input sensing for plants to distinguish between the different types of low energy and carbon stresses requiring different output strategies that control glycolysis, TCA or even mitochondrial oxidative phosphorylation separately as previously observed in the case of yeast Snf1 (De Virgilio, 2011). This input sensing may, in part, be mediated by the plant TPS with no apparent catalytic activity. It is clear that plants differentially activate the expression of genes from the T6P metabolism in response to extended darkness, submergence, and hypoxia, for example (Lee et al., 2011).

Concluding remarks

The TPS/T6P/SnRK1 regulation mechanism is unlikely to be the only carbon/energy sensing and signal transduction system in plants, but its effects are major. The mechanism probably evolved from an ancient pathway. It is found in fungi where it, along with the Target of Rapamycin 1 (TORC1), Protein Kinase A (PKA), and Cyclin Dependent Kinase CDK Pho85, controls the quiescent state of yeast for example (De Virgilio, 2011). T6P constitutes the first documented primary metabolite that controls metabolism and, therefore, growth in plants. It is found at very low concentrations that are comparable with hormones. Because of its charged nature it is assumed that its action is cell autonomous.

Research is needed on all fronts to further our embryonal understanding of the components in the system. Biochemical studies have been lacking, in particular to understand the properties of the enzymes from the large families of plant TPS and TPP. Since we will probably have to content ourselves with the tedious bucket chemistry approach to evaluate the low T6P quantities found in plant tissues in the near future, genetic approaches will be required to dissect the effects of this regulatory primary metabolite with higher resolution. An understanding of the transport across subcellular compartments is lacking for T6P whilst it is thought that TpE may be transported by Suc transporters.

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