SnRK1 and trehalose 6-phosphate – two ancient pathways converge to regulate plant metabolism and growth

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Abstract

SUCROSE-NON-FERMENTING1-RELATED KINASE1 (SnRK1) belongs to a family of protein kinases that originated in the earliest eukaryotes and plays a central role in energy and metabolic homeostasis. Trehalose 6-phosphate (Tre6P) is the intermediate of trehalose biosynthesis, and has even more ancient roots, being found in all three domains of life – Archaea, Bacteria and Eukarya. In plants, the function of SnRK1 has diverged from its orthologues in fungi and animals, evolving new roles in signalling of nutrient status and abiotic stress. Tre6P has also acquired a novel function in plants as a signal and homeostatic regulator of sucrose, the dominant sugar in plant metabolism. These two ancient pathways have converged in a unique way in plants, enabling them to coordinate their metabolism, growth and development with their environment, which is essential for their autotrophic and sessile lifestyle.

Introduction

SUCROSE-NON-FERMENTING1-RELATED KINASE1 (SnRK1) belongs to a family of Ca^{2+}-independent serine/threonine protein kinases that is represented by the Sucrose-non-fermenting1 (SNF1) kinase in yeast and other fungi, and by the AMP-activated kinase (AMPK) in animals [1,2]. They function as heterotrimeric complexes comprising an α-catalytic subunit and two regulatory subunits (β and γ) and are activated under low-energy conditions to restore energy homeostasis (Fig. 1). This is achieved by simultaneous down-regulation of anabolic processes (e.g. protein synthesis and cell proliferation) and up-regulation of catabolic processes (e.g. autophagy). While retaining this fundamental role, their targets and regulation have diverged between fungi, animals and plants, reflecting the different lifestyles of these organisms. In plants, SnRK1 has acquired particular importance in nutrient sensing and stress responses, and plays a central role in coordinating the growth and development of plants with their environment [3-5].

Trehalose 6-phosphate (Tre6P) is the intermediate in the two-step pathway of trehalose biosynthesis mediated by Tre6P-synthase (TPS) and Tre6P-phosphatase (TPP), which occurs in the Archaea, Bacteria and Eukarya, including plants [6]. Trehalose has multiple roles in prokaryotes, fungi and invertebrates: osmolyte, carbon reserve, transport sugar and stress protectant, and is likely to have similar functions in non-vascular plants, sharing these with another disaccharide, sucrose. The evolution of vascular tissues – phloem and xylem – and adoption of sucrose as the major transport sugar, led to sucrose becoming the dominant sugar in higher-plant metabolism and making trehalose redundant [7]. It has been speculated that this freed Tre6P and trehalose to evolve new functions as signal molecules, with Tre6P becoming a signal [8] and regulator of sucrose status [9,10], as described in the sucrose-Tre6P nexus model (Fig. 2), while trehalose is implicated in plant interactions with beneficial micro-organisms and pathogens [11,12].

In the last 10-15 years, multiple connections between the SnRK1 and Tre6P signalling pathways in higher plants have been identified, indicating that these ancient pathways have converged. In this review, we discuss recent discoveries about the molecular mechanisms connecting the SnRK1 and Tre6P signalling pathways and their physiological significance.

Regulation of Tre6P signalling by SnRK1

Among the first SnRK1 targets to be identified in plants were central metabolic enzymes, including sucrose-phosphate synthase (SPS), nitrate reductase (NR) and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase [1,13,14]. Further targeted and untargeted studies [15-17] have identified many more target proteins including additional enzymes of C metabolism [18], enzymes and transcriptional regulators of lipid metabolism [19-22], bZIP transcription factors [23-26] and translation initiation factors [27,28]. In Arabidopsis (*Arabidopsis thaliana*), several non-catalytic (class II) isoforms of TPS-like proteins (AtTPS5-AtTPS7) have been identified as SnRK1 targets [15,16,29], with phosphorylation of the proteins leading to association with 14-3-3 proteins [29]. However, it is unclear whether association with 14-3-3s promotes or inhibits protein functions or controls their degradation via the ubiquitin-26S proteasomal pathway [1]. SnRK1 is also involved in the transcriptional regulation of TPS-like isoforms [30], in a manner that requires the bZIP11 transcription factor [24]. In Arabidopsis, AtTPS5 is implicated in thermotolerance [12] and ABA signalling [31], AtTPS6 in regulation of cell size and shape [32] and TPS11 in aphid resistance [33]. In other species, orthologues of AtTPS8 and AtTPS11 have been implicated in salt and cold stress responses, respectively [34,35]. However, the molecular functions of the class II TPS-like proteins are poorly understood, so the physiological significance of their transcriptional regulation and post-translational modification by SnRK1 is unclear.

Regulation of SnRK1 by Tre6P – evidence from *in-vitro* studies and gene expression

Early studies using partly purified extracts already showed an inhibitory effect of sugar phosphates, namely G6P, on SnRK1 activity [13]. However, it was only much later that a similar effect was described for Tre6P [36]. This work showed that SnRK1 activity is inhibited by micromolar amounts of Tre6P *in vitro* in desalted extracts of actively growing tissues but not in extracts of mature leaves. Sensitivity to Tre6P was lost in immunoprecipitated SnRK1 but could be restored by supplementation with extracts from seedlings. Heat denatured extracts were no longer able to mediate SnRK1 repression by Tre6P, indicating the involvement of a proteinaceous factor [36]. Other sugars such as G6P and G1P also inhibit SnRK1 in young tissues, possibly *via* the same intermediary protein factor [37]. Very recent work, demonstrated that Tre6P acts at least partly through the SnRK1 activating kinases SnAK2 and SnAK1 [21] (also known as GRIK1 and GRIK2, respectively). Tre6P binds directly to SnRK1 at a site different to that of ATP, diminishing the interaction with the SnAKs and thereby SnRK1 phosphorylation and activity. Unexpectedly, Tre6P stimulated SnRK1 activity in extracts of the double *snak1snak2* mutant, suggesting a dual function of Tre6P in regulation of SnRK1 that is dependent on the presence or absence of SnAKs [21]. The SnAKs appear to

be widely expressed, including in mature leaves (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi;), suggesting that they are not the intermediary factor described by Zhang and colleagues, and that there may be multiple mechanisms of interaction between Tre6P and SnRK1.

In addition to Arabidopsis plants at different developmental stages [36,38-40], inhibition of SnRK1 activity by Tre6P in desalted extracts has also been reported in young spinach leaves, broccoli florets [36,38-40], potato tubers [41], developing wheat grains [42], developing bean seeds [43], cauliflower curd [36,40], cucumber peduncles [44], maize ear spikelets [45], spring wheat [46], and *Setaria viridis* [47]. Although the degree to which Tre6P represses SnRK1 activity varies depending on the species and tissue, it is generally greater in young and actively growing organs. One caveat for *in-vitro* studies is the low purity of some commercial sources of Tre6P [8,10], so if the purity of Tre6P was not checked, there is a theoretical possibility that contaminants may have contributed to the apparent inhibitory effects of Tre6P *in vitro*.

Changes in gene expression in Arabidopsis have been interpreted as evidence of a repressive effect of Tre6P on SnRK1 *in vivo*, with genes that are normally induced by SnRK1 being repressed when Tre6P levels are high and genes that are normally repressed by SnRK1 being induced by Tre6P [30,36]. Similar overlaps in gene expression profiles have been reported in other species in several tissues and organs [39,41,42,46,48,49], and under conditions of stress [48,50,51]. Intriguingly, in most of these cases, changes in SnRK1 marker gene expression are not accompanied by clear changes in total SnRK1 activities determined by *in-vitro* peptide phosphorylation assays [36,39,41,42,46,49]. This may indicate loss of regulatory factors during the extraction process or the observed downstream changes in gene expression being driven by only a fraction of the total SnRK1 pool. This fraction may remain undetectable when assaying total SnRK1 activities or may be linked to *in-viv*o changes in SnRK1 localization that are lost upon extraction.

Even more surprisingly, SnRK1 marker gene expression follows Tre6P levels even when total SnRK1 activities are dramatically reduced, as shown during the initial response of maize kernels upon excision and incubation on medium without sucrose (starved kernels; [48]). On the other hand, 48 h after the excision and, despite having similarly low Tre6P levels, starved kernels showed higher expression of SnRK1 marker genes than kernels supplemented with sucrose. Together these observations suggest that gene expression is not always a faithful readout of SnRK1 activity or Tre6P-SnRK1 interactions *in vivo*, as phosphorylation by SnRK1 might not be the only form of regulation of its target transcription factors and the genes they control. Furthermore, constitutive or long-term changes in Tre6P levels (e.g. in *35S:otsA* or *35S:otsB* plants; [36] lead to large and opposite changes in sucrose [10], which can affect gene expression in a Tre6P-independent manner (e.g. *via* translational regulation of bZIP11).

Regulation of SnRK1 by Tre6P in source leaves

In most higher plants, sucrose is the main product of photosynthesis and is exported from the leaves via the phloem to provide carbon and energy for growing sink organs [7]. In source

leaves, the major function of Tre6P is to regulate sucrose production to balance this with demand for sucrose. In the light, Tre6P modulates the partitioning of photoassimilates between sucrose and organic and amino acids, via post-translational activation of phospho*enol*pyruvate carboxylase (PEPC) and NR [52]. SnRK1 is one of the protein kinases that phosphorylates NR, and the activation of NR in plants with elevated Tre6P is consistent with inhibition of SnRK1 by Tre6P [52]. However, no changes were observed in the activation state of SPS, which is often used as readout of SnRK1 activity. Likewise, there were no changes in levels of fructose-2,6-bisphosphate, which is synthesized and dephosphorylated by another SnRK1 target protein – the bifuctional F2KP enzyme [52]. Thus, the involvement of SnRK1 in downstream responses to Tre6P in illuminated source leaves remains unclear.

At night, Tre6P regulates the remobilization of transitory starch reserves into sucrose [9,12,53], but the molecular mechanisms involved are not yet understood. It has been observed that the diurnal profile of starch synthesis and degradation is altered in various *snrk1* mutants (see [54] and references therein); however, these results must be interpreted with caution as perturbation of SnRK1 activity has pleiotropic effects on growth that could affect starch metabolism indirectly. Starch degradation is under the control of the circadian clock, especially under short-day conditions. The recent discovery that SnRK1 is involved in transcriptional regulation of core components of the circadian clock suggests one way in which SnRK1 might influence starch degradation [23,55]. The SnRK1βγ subunit contains multiple carbohydrate binding modules that are implicated in binding to starch and regulation of SnRK1 activity by maltose [1,56]. Thus, there are several potential mechanisms by which SnRK1 could regulate starch metabolism in source leaves, but further work is needed to clarify how these operate *in vivo*, and whether regulation of SnRK1 by Tre6P is a significant factor.

Regulation of SnRK1 by Tre6P in sink organs

The sensitivity of SnRK1 activity to Tre6P is mostly detected *in vitro* in extracts from actively growing tissues [36], such as young seedlings, developing seeds and tubers, and it is in these tissues that Tre6P-SnRK1 interactions are likely to have the greatest importance *in vivo*. When considering the downstream effects of the Tre6P-SnRK1 interactions, it is important to distinguish between control of a particular developmental transition and subsequent growth once the decision to progress to the next developmental stage has been made. In general terms, high Tre6P levels and/or low SnRK1 activities are associated with developmental progression, whilst low Tre6P levels and/or high SnRK1 activities are associated with a dormant state. Acting as a signal of sucrose availability in the shoot apical meristem and axillary buds, Tre6P promotes various developmental transitions, including flowering and shoot branching [9,57,58]. Conversely, overexpression of SnRK1 delays germination and flowering [3].

SnRK1 generally acts as a brake on development, but once a developmental transition is initiated, SnRK1 activity is required to support the growth of the new sinks. This may be accomplished by promoting nutrient remobilization from the source tissues, by directly modulating nutrient import and growth of the sink, or both. For example, OtsB (TPP)

overexpressing potato tubers accumulated higher levels of glucose and sucrose and displayed premature sprouting [41], whereas antisense *snrk1* tubers had strongly delayed sprouting, suggesting that reduced Tre6P levels cause increased reserve mobilisation via SnRK1 signalling. Furthermore, SnRK1 is required for adequate nutrient remobilization and supply to the growing sinks during rice germination, and this was proposed to confer a particular advantage under low oxygen conditions [13]. Consistent with this, rice plants expressing OsTPP7 have enhanced capacity to remobilize starch reserves from the seed and provide nutrients to the rapidly elongating coleoptile, a trait that is highly beneficial under submergence [59]. Interestingly, despite leading to clear phenotypes, altered expression of TPP enzymes is not always accompanied by an obvious reduction in Tre6P levels [10,59]. This may be due to changes in Tre6P levels being localized to specific cell or tissue types, which might not be detected in metabolite analyses of whole-plant or whole-organ samples (discussed in [60]). Additionally, TPPs can have "moonlighting" functions that are independent of their catalytic activity [61] [39].

Several studies indicate that Tre6P and inhibition of SnRK1 activity are necessary for the activation of biosynthetic processes in growing tissues in response to sucrose supply. Tre6P promotes the utilization of sucrose for growth, for example, by coordinating changes in central metabolism to provide the building blocks for cell division and expansion [57], and the accumulation of storage reserves [21,22,41,48,50,51]. Induction of hypocotyl elongation in response to sucrose or elevated ambient temperatures is defective in *tps1* mutants with weak (non-lethal alleles) and in plants overexpressing SnRK1α1, further supporting the idea that suppression of SnRK1 activity by T6P is necessary to allow cell elongation [62,63]. Effects of sucrose on entrainment of the circadian clock, which regulates many processes including hypocotyl growth, could also be dependent on inhibition of SnRK1 by Tre6P [23].

Conversely, spraying spring wheat plants or ears during the grain filling period with membrane permeable Tre6P analogues enhanced growth and yield under normal field conditions and during mild drought, and this was accompanied by decreased SnRK1 signaling [46]. A beneficial effect of sucrose and Tre6P accumulation was also shown under conditions of sink limitation (e.g cold stress). Inhibition of SnRK1 by Tre6P appears to be important for the efficient resumption of growth once favorable conditions are restored [39]. Preferential growth of the first fruit in cucumber was also found to be associated with a higher accumulation of sucrose and Tre6P levels and with a lower SnRK1 activity than in the second fruit [44].

These seemingly contradictory results may be attributable to the highly dynamic nature of the sucrose-Tre6P-SnRK1 relationship and involvement of each component in separate, as well as linked, signalling pathways. Both repression and activation of SnRK1 may be required at different phases of growth and development and in different tissues to allow developmental progression and growth. This strongly highlights the need to increase the temporal and spatial resolution of analyses when studying Tre6P and SnRK1 signalling, and to develop non-invasive techniques that allow *in-vivo* monitoring of Tre6P accumulation and SnRK1 activity.

Conclusions

- SnRK1 is regulated by multiple mechanisms, including inhibition by Tre6P via interaction with SnAKs(GRIKs) and via unknown protein factors (see Fig. 1; [1,56,64-67]
- Despite being generally considered a growth suppressor, SnRK1 is required for proper development, as complete suppression of SnRK1 activity is detrimental to growth.
- To better understand Tre6P-SnRK1 interactions, more work is required with reconstituted *in-vitro* systems [68], improved readouts of Tre6P and SnRK1 function *in vivo*, and cell/tissue specific manipulations of Tre6P and SnRK1.

Conflict of interest statement

Declaration of interest: none.

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●● Hypocotyl elongation in response to elevated ambient temperature is shown to be dependent on the control of PIF4 stability by the T6P-SnRK1 axis. The inability of a tps1 mutant to elongate the hypocotyl is suppressed when SnRK1 activity is depleted in this background, providing for the first time genetic evidence that T6P acts via SnRK1.

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● This study reports a central role of TPS1-SnRK1 in sucrose-induced hypocotyl elongation, linking these components to gibberellin and auxin signalling.

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● This study provides new information on the localization and intracellular movement of SnRK1. One interesting feature is the localization to the ER and that relocalization to the nucleus upon stress may be more prominent in specific tissues such as the vasculature

65. Jamsheer KM, Jindal S, Laxmi A: **Evolution of TOR-SnRK dynamics in green plants and its integration with phytohormone signaling networks**. *J Exp Bot* 2019, **70**:2239-2259.

●● Excellent review on the evolution of the SnRK family (SnRK1s, SnRK2s, and SnRK3s) in plants and their increasing connections to the growth-promoting TOR kinase and hormone signalling pathways.

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●● This study reports novel mechanisms for SnRK1 activation and re-localization. Several lines of evidence are presented to demonstrate that SnRK1α1 has regulatory subunit-independent activity. Furthermore, SnRK1 relocalizes to the nucleus in response to energy stress, with myristoylation of the beta subunits restricting this relocalization. Each of these

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Figure legends

Figure 1. SnRK1 subunit composition and domain architecture.

Comparison of SnRK1 subunits with their opisthokont counterparts reveals plant-specific subunits and domain structure. Mammalian AMPK subunits are shown as representatives of the opisthokont kinases. The α-subunit (in blue) is composed of an N-terminal catalytic and a C-terminal regulatory domain. The catalytic domain harbors the T-loop, which requires phosphorylation on the indicated conserved threonine for kinase activity. The regulatory domain contains an autoinhibitory domain (AID) in AMPK and a ubiquitin-associated (UBA) domain in plants. Contrary to the repressive effect of the AID in AMPK, the UBA domain appears to promote SnRK1 phosphorylation by its upstream kinase and to sustain its catalytic activity [69]. The regulatory domain contains also the C-terminal region that is important for interaction with the regulatory subunits and other proteins. The main catalytic subunits in Arabidopsis are SnRK1α1 and SnRK1α2, with SnRK1α3 being only expressed at low levels in pollen and seeds. All β-subunits (in grey) harbor a C-terminal region for interaction with the other subunits of the complex. Typically, they also contain an N-terminal glycine that is myristoylated (Myr), and a carbohydrate binding domain (CBM). The plant β3 subunit is

unique in lacking these two features. The γ-subunit (in red) harbors four CBS (cystathionine-β-synthase) domains that in AMPK bind adenylates and regulate kinase complex conformation and activity in response to energy signals. The plant γ subunit has a unique structure, having integrated features both of β- (CBM domain) and γ-subunits (CBS domains). Modeling the CBS of SnRK1βγ using the AMPKγ structure identified amino acid substitutions that may disrupt adenylate binding at the nucleotide-binding sites [69], providing an explanation for the lack of allosteric regulation by adenylates and for their effect in protecting from phosphatases. For more details on the structure of the subunits and their regulation see [1,65].

Figure 2. The sucrose-Tre6P nexus in source and sink organs.

The sucrose-Tre6P nexus model postulates that Tre6P functions as both a signal and negative feedback regulator of sucrose levels in plant tissues, contributing to sucrose homeostasis in a manner that has been likened to the control of blood glucose by insulin [10]. It is proposed that sucrose positively regulates the level of Tre6P by activation of Tre6P synthase (TPS) or inhibition of Tre6P phosphatase (TPP), or both. In source leaves (A), Tre6P modulates sucrose levels mainly by regulating sucrose synthesis. During the day, Tre6P regulates the partitioning of photoassimilates between sucrose and organic and amino acids, via post-translational modulation of phosph*enol*pyruvate carboxylase (PEPC) and nitrate reductase (NR). At night, Tre6P regulates the remobilization of transitory starch reserves to sucrose (not shown). Connected to the core nexus are signalling pathways downstream of Tre6P that regulate secondary metabolism (e.g. anthocyanin synthesis) and developmental processes (e.g. flowering and axillary bud outgrowth). There are also Tre6P-idependent pathways of sucrose signalling. In sink organs (B), such as developing seeds, Tre6P modulates sucrose levels mainly by regulating its utilization for growth, in part via inhibition of SnRK1. There is evidence for multiple mechanisms by which Tre6P inhibits SnRK1; it can bind to the α (catalytic) subunit, thereby inhibiting activation of SnRK1 by SnRK1 activating kinase (SnAK) [21], or it can inhibit SnRK1 via association with an unknown protein factor (X) [36].

Figure 3. Regulation of SnRK1 by Tre6P and other factors.

SnRK1 is primarily activated by low energy conditions, often associated with stress. When activated, it helps to restore energy status by repressing anabolic processes (e.g. protein synthesis) that are needed for growth, and stimulating catabolic processes including

autophagic turnover of proteins and other cellular components. SnRK1 is regulated by multiple mechanisms. Phosphorylation of a conserved threonine residue (Thr175 in Arabidopsis) in the α (catalytic) subunit is necessary for activation of SnRK1 and this may involve autophosphorylation [66]. Conversely, SnRK1 may be ubiquitinated and degraded via the 26S proteasome through the action of different components, including the SIZ1 SUMO E3 ligase, the SnRK1-interacting DWD protein PRL1, the splicing factor SR45, and the myo-inositol polyphosphate 5-phosphatase 5PTase13. Proteasomal degradation of SnRK1 may be associated with change in the subunit composition of the trimeric SnRK1 complexes, with assembly of new complexes being mediated by FCS-like zinc finger (FCSL-ZF) proteins [65]. SnRK1 is also activated allosterically by maltose binding to carbohydrate-binding modules in the α-subunits (see Fig. 1), suggesting a role for SnRK1 in regulating the remobilization of transitory starch to sucrose [56]. SnRK1 localization is affected by myristoylation of the SnRK1β1 and β2 subunits, which target SnRK1 complexes e.g. to the endoplasmic reticulum [66,67]. Conversely, low energy signals induce SnRK1 translocation to the nucleus [64,66]. It is not yet known if Tre6P is involved in any of these regulatory mechanisms, however, there are at least two other regulatory mechanisms that do involve Tre6P. The SnRK1 complex from developing tissues is inhibited by Tre6P (also glucose 6-phosphate and glucose 1-phosphate) in a manner that is dependent on an unidentified protein factor (Protein X) [36]. SnRK1 activating kinases (SnAKs, also known as GRIKs) activate SnRK1 by phosphorylation, and this post-translation activation process is inhibited by binding of Tre6P to the α (catalytic) subunit of SnRK1 [21]. Phosphorylation of SnRK1 by SnAKs may be counteracted by the action of protein phosphatase type 2C (PP2C), but it is not yet known if this is also influenced by Tre6P. ABA signals further contribute to SnRK1 signaling through inhibition of PP2Cs. For further details on these mechanisms please see [1,65].

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Figure

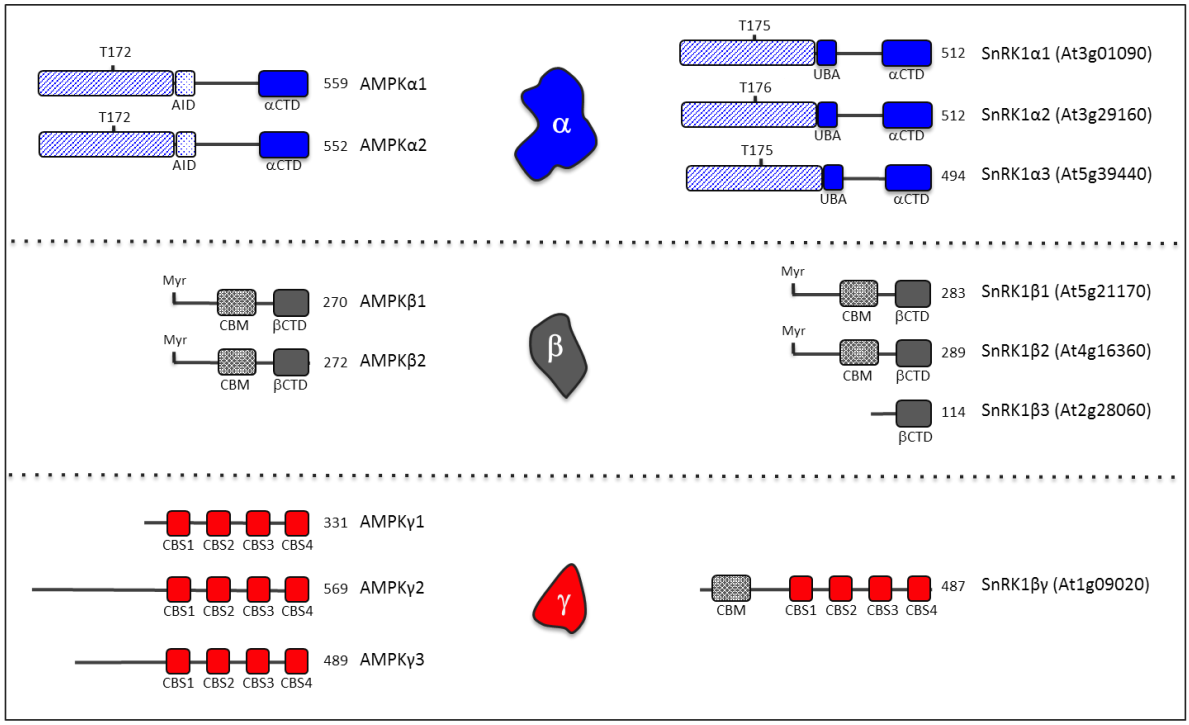

Figure 1. SnRK1 subunit composition and domain architecture.

Comparison of SnRK1 subunits with their opisthokont counterparts reveals plant-specific subunits and domain structure. Mammalian AMPK subunits are shown as representatives of the opisthokont kinases. The α-subunit (in blue) is composed of an N-terminal catalytic and a C-terminal regulatory domain. The catalytic domain harbors the T-loop, which requires phosphorylation on the indicated conserved threonine for kinase activity. The regulatory domain contains an autoinhibitory domain (AID) in AMPK and an ubiquitin-associated (UBA) domain in plants. Contrary to the repressive effect of the AID in AMPK, the UBA domain appears to promote SnRK1 phosphorylation by its upstream kinase and to sustain its catalytic activity [Emanuelle et al. 2018]. The regulatory domain contains also the C-terminal region that is important for interaction with the regulatory subunits and other proteins. The main catalytic subunits in Arabidopsis are SnRK1α1 and SnRK1α2, with SnRK1α3 being only expressed at low levels in pollen and seeds. All β-subunits (in grey) harbor a C-terminal region for interaction with the other subunits of the complex. Typically they also contain an N-terminal glycine that is myristoylated (Myr), and a carbohydrate binding domain (CBM). The plant β3 subunit is unique in lacking these two features. The γ-subunit (in red) harbors four CBS (cystathionine-β-synthase) domains that in AMPK bind adenylates and regulate kinase complex conformation and activity in response to energy signals. The plant γ subunit has a unique structure, having integrated features both of β- (CBM domain) and γ-subunits (CBS domains). Modeling the CBS of SnRK1βγ using the AMPKγ structure identified amino acid substitutions that may disrupt adenylate binding at the nucleotide-binding sites [Emanuelle et al. 2015], providing an explanation for the lack of allosteric regulation by adenylates and for their effect in protecting from phosphatases. For more details on the structure of the subunits and their regulation see [1] [63].

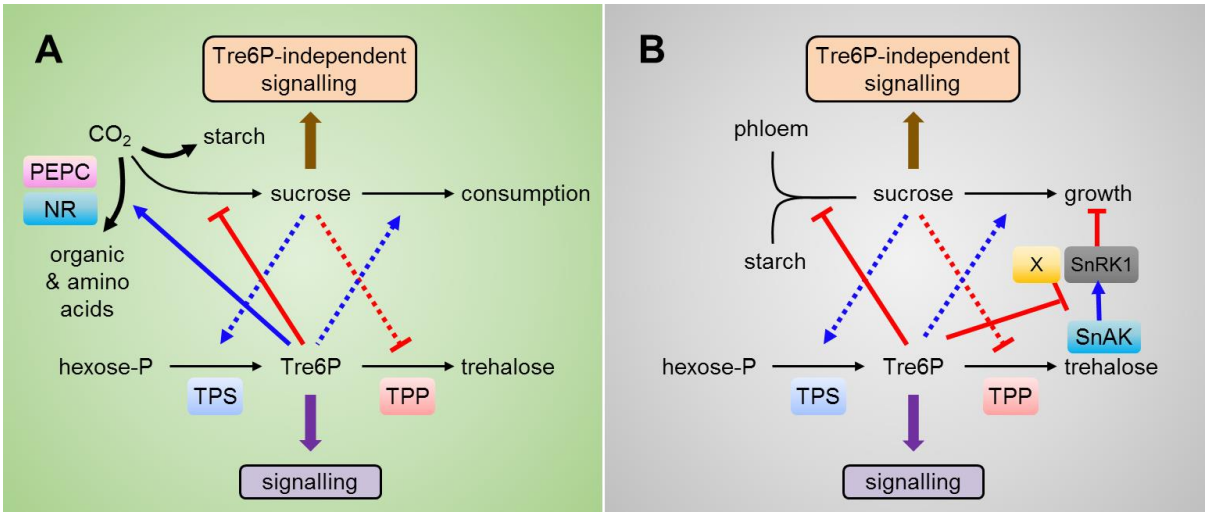

Figure 2. The sucrose-Tre6P nexus in source and sink organs.

The sucrose-Tre6P nexus model postulates that Tre6P functions as both a signal and negative feedback regulator of sucrose levels in plant tissues, contributing to sucrose homeostasis in a manner that has been likened to the control of blood glucose by insulin (Yadav et al., 2014). It is proposed that sucrose positively regulates the level of Tre6P by activation of Tre6P synthase (TPS) or inhibition of Tre6P phosphatase (TPP), or both. In source leaves (A), Tre6P modulates sucrose levels mainly by regulating sucrose synthesis. During the day, Tre6P regulates the partitioning of photoassimilates between sucrose and organic and amino acids, via post-translational modulation of phosph*enol*pyruvate carboxylase (PEPC) and nitrate reductase (NR). At night, Tre6P regulates the remobilization of transitory starch reserves to sucrose (not shown). Connected to the core nexus are signalling pathways downstream of Tre6P that regulate secondary metabolism (e.g. anthocyanin synthesis) and developmental processes (e.g. flowering and axillary bud outgrowth). There are also Tre6P-idependent pathways of sucrose signalling. In sink organs (B), such as developing seeds, Tre6P modulates sucrose levels mainly by regulating its utilization for growth, in part via inhibition of SnRK1. There is evidence for multiple mechanisms by which Tre6P inhibits SnRK1; it can bind to the a (catalytic) subunit, thereby inhibiting activation of SnRK1 by SnRK1 activatining kinase (SnAK) [21], or it can inhibit SnRK1 via association with an unknown protein factor (X) [36].

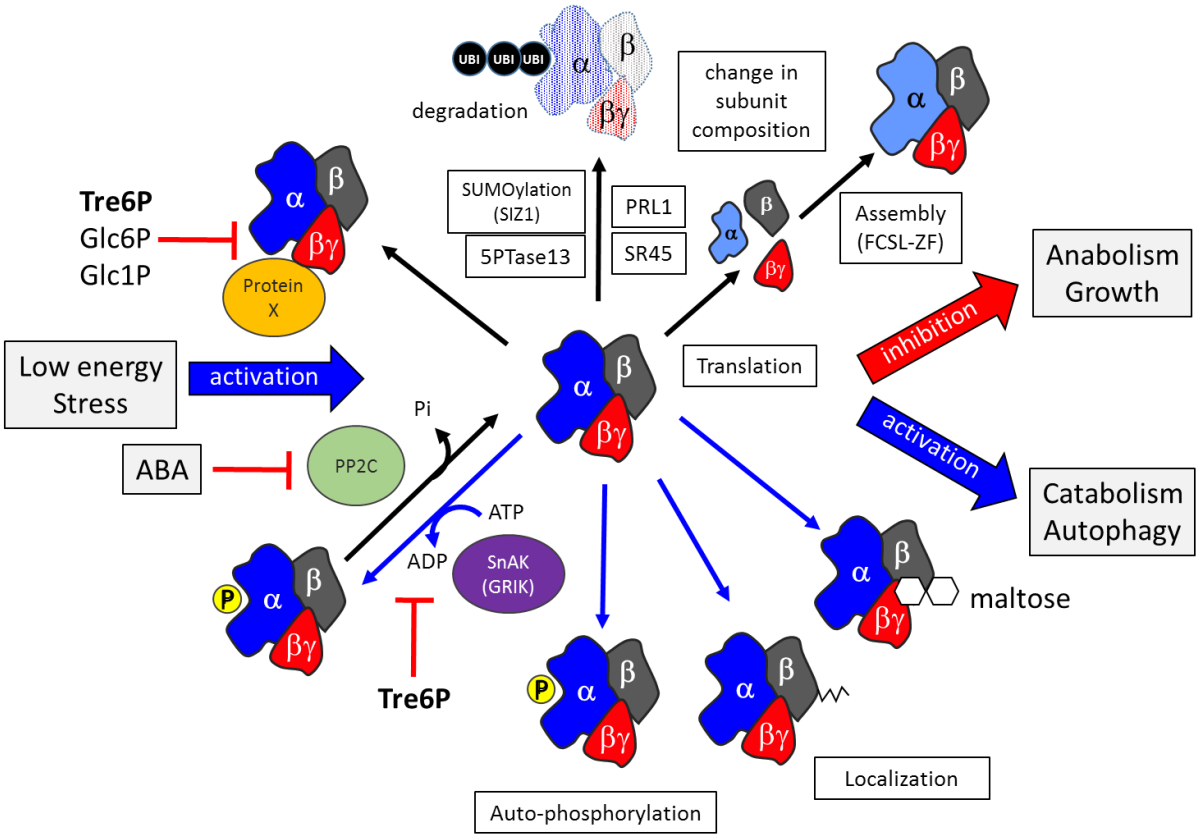

Figure 3. Regulation of SnRK1 by Tre6P and other factors.

SnRK1 is primarily activated by low energy conditions, often associated with stress. When activated, it helps to restore energy status by repressing anabolic processes (e.g. protein synthesis) that are needed for growth, and stimulating catabolic processes including autophagic turnover of proteins and other cellular components. SnRK1 is regulated by multiple mechanisms. Phosphorylation of a conserved threonine residue (Thr175 in Arabidopsis) in the α (catalytic) subunit is necessary for activation of SnRK1 and this may involve autophosphorylation [64]. Conversely, SnRK1 may be ubiquitinated and degraded via the 26S proteasome through the action of different components, including the SIZ1 SUMO E3 ligase, the SnRK1-interacting DWD protein PRL1, the splicing factor SR45, and the myo-inositol polyphosphate 5-phosphatase 5PTase13. Proteasomal degradation of SnRK1 may be associated with change in the subunit composition of the trimeric SnRK1 complexes, with assembly of new complexes being mediated by FCS-like zinc finger (FCSL-ZF) proteins [63]. SnRK1 is also activated allosterically by maltose binding to carbohydrate-binding modules in the α-subunits (see Fig. 1), suggesting a role for SnRK1 in regulating the remobilization of transitory starch to sucrose [55]. SnRK1 localization is affected by myristoylation of the SnRK1β1 and β2 subunits, which target SnRK1 complexes e.g. to the endoplasmic reticulum [64] [65]. Conversely, low energy signals induce SnRK1 translocation to the nucleus [62] [64]. It is not yet known if Tre6P is involved in any of these regulatory mechanism, however, there are at least two other regulatory mechanisms that do involve Tre6P. The SnRK1 complex from developing tissues is inhibited by Tre6P (also glucose 6-phosphate and glucose 1-phosphate) in a manner that is dependent on an unidentified protein factor (Protein X) [36]. SnRK1 activating kinases (SnAKs, also known as GRIKs) activate SnRK1 by phosphorylation, and this

post-translation activation process is inhibited by binding of Tre6P to the α (catalytic) subunit of SnRK1 [21]. Phosphorylation of SnRK1 by SnAKs may be counteracted by the action of protein phosphatase type 2C (PP2C), but it is not yet known if this is also influenced by Tre6P. ABA signals further contribute to SnRK1 signaling through inhibition of PP2Cs. For further details on these mechanisms please see [1] [63].