REVIEW PAPER

SNF1-related protein kinase 1: the many-faced signaling hub regulating developmental plasticity in plants

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Abstract

The Snf1-related protein kinase 1 (SnRK1) is the plant homolog of the heterotrimeric AMP-activated protein kinase/sucrose non-fermenting 1 (AMPK/Snf1), which works as a major regulator of growth under nutrient-limiting conditions in eukaryotes. Along with its conserved role as a master regulator of sugar starvation responses, SnRK1 is involved in controlling the developmental plasticity and resilience under diverse environmental conditions in plants. In this review, through mining and analyzing the interactome and phosphoproteome data of SnRK1, we are highlighting its role in fundamental cellular processes such as gene regulation, protein synthesis, primary metabolism, protein trafficking, nutrient homeostasis, and autophagy. Along with the well-characterized molecular interaction in SnRK1 signaling, our analysis highlights several unchartered regions of SnRK1 signaling in plants such as its possible communication with chromatin remodelers, histone modifiers, and inositol phosphate signaling. We also discuss potential reciprocal interactions of SnRK1 signaling with other signaling pathways and cellular processes, which could be involved in maintaining flexibility and homeostasis under different environmental conditions. Overall, this review provides a comprehensive overview of the SnRK1 signaling network in plants and suggests many novel directions for future research.

Keywords: Nutrient sensing, plant development, protein kinase, regulatory hub, SnRK1, stress response, sugar signaling.

Introduction

The sensing of nutrient status and adjusting growth is critical for the survival of organisms. Sugars, amino acids, and minerals are vital for cell maintenance, growth, and division. Nutrient sufficiency promotes growth and cell division, whereas nutrient deficiency imposes restrictions on growth, and promotes nutrient recycling to help cells survive. When the nutrients become available in the environment, cells can rapidly sense them and accelerate growth. Cells possess distinct sensors of nutrients which help in coordinating growth according to nutrient availability. Both eukaryotes and prokaryotes possess distinct as well as common nutrient sensors (Chantranupong et al., 2015). In eukaryotes, a serine-threonine kinase named AMP-activated protein kinase (AMPK) works as a sensor of nutrient starvation (Steinberg and Kemp, 2009). Although AMPK and its homologs were initially described to regulate the growth during energy (sugar) starvation, recent studies found that it also responds to amino acid and mineral nutrient levels (Orlova et al., 2006; Davie et al., 2015; Dalle Pezze et al., 1996).
AMPK was initially identified as a kinase that regulates the activity of enzymes involved in lipid biosynthesis in the liver (Steinberg and Kemp, 2009). The budding yeast homolog of AMPK, named sucrose non-fermenting 1 (Snf1), was identified from a forward genetic screen of mutants defective in utilizing sucrose as the carbon source (Carlson et al., 1981). Extensive studies on mammalian and yeast systems in the last few decades have established it as a regulator of growth during nutrient starvation (Hardie, 2018). AMPK/Snf1 works as an obligate heterotrimer with catalytic α and regulatory β and γ subunits (Fig. 1). Interestingly, a recent study identified that unlike AMPK/Snf1, Arabidopsis Snf1-related protein kinase 1 (SnRK1) kinase subunit possesses regulatory subunit-independent SnRK1 activity in regulating the expression of target genes (Ramon et al., 2019).

AMPK works as a sensor of several types of nutrients in eukaryotes. Similar to other kinases, the phosphorylation at Thr172 in the activation loop (T-loop) of the α subunit in the catalytic domain (CD) is critical for AMPK activity (Fig. 1A). AMPK activity is highly correlated with T-loop phosphorylation (Herzig and Shaw, 2018; Lin and Hardie, 2018). Although essential for the function, a clear correlation between activity and T-loop phosphorylation is lacking in the case of SnRK1 (Baena-González et al., 2007; Fragoso et al., 2009; Rodrigues et al., 2013; Emanuelle et al., 2015). Thr172 phosphorylation occurs through upstream activating kinases such as liver kinase B1 (LKB1) (Oakhill et al., 2011). LKB1 homologs are present in several eukaryotic lineages, including fungi and plants. In plants, LKB1 homologs are named SnRK1 activating kinase 1 and 2 (SnAK1 and 2) (Shen et al., 2009). Studies in Arabidopsis indicate that the SnAKs might be working as major upstream activating kinases of SnRK1 in plants. In in vitro assays, SnAKs were found to be crucial in activating SnRK1α by

Fig. 1. Representative domain composition of SnRK1/AMPK/Snf1 subunits from different plant species in comparison with other eukaryotic supergroups. (A–C) The domain composition of α kinase and β, γ, βγ regulatory subunits. The different eukaryotic supergroups are distinguished by colors. The protein sequences were retrieved through BLAST searches using Arabidopsis SnRK1 subunits as the query. IBS webserver was used to annotate domains utilizing the information obtained from CD-search (CDD v3.18 database) and InterProScan (InterPro v79.0 database). (D) The phylogram of the species used for domain composition analysis was created using TimeTree.
phosphorylating the conserved threonine residue in the T-loop (Shen et al., 2009; Crozet et al., 2010). However, expression of SnAKs is limited to young and dividing tissues, and is enhanced during viral infection (Shen and Hanley-Bowdoin, 2006; Shen et al., 2009). Conversely, the phosphorylated form of SnRK1α can be detected in mature tissues as well (Shen et al., 2009). Therefore, the restricted expression pattern of SnAKs and lack of correlation between T-loop phosphorylation and SnRK1 activity suggests the possible existence of other more important mechanisms regulating the spatiotemporal SnRK1 activity in plants. Nonetheless, in line with the in vitro evidence, a later study identified that loss of both SnAKs leads to a strong decrease in the level of T-loop phosphorylation of SnRK1α in planta despite having a comparable protein level (Glab et al., 2017). Thus, the regulation of AMPK/Snf1/SnRK1 activity by upstream kinases is conserved in eukaryotes. Interestingly, activated SnRK1 directly phosphorylates SnAKs which negatively regulates SnAK activity in in vitro assays (Crozet et al., 2010). This phosphorylation might be working as a feedback regulatory mechanism for controlling SnRK1 signaling. However, the biological significance of this phosphorylation is yet to be established. AMPK and Snf1 are negatively regulated by protein phosphatases by dephosphorylating the conserved threonine residue in the T-loop of the kinase subunit (Crozet et al., 2014). Members of the protein phosphatase 2C (PP2C) family inactivates SnRK1 using a similar mechanism in plants (Rodrigues et al., 2013).

Studies in plant systems, especially in Arabidopsis, identified that in comparison with AMPK/Snf1, SnRK1 shows key differences in subunit composition and the regulation of activity. Nonetheless, SnRK1 works as a critical regulator of the plant’s response towards extended darkness and sugar starvation (Baena-González et al., 2007; Mair et al., 2015; Nukarinien et al., 2016; Pedrotti et al., 2018). Thus, it appears that the function of AMPK/Snf1/SnRK1 as a universal regulator of sugar starvation responses pre-dates the divergence of eukaryotes. Further, the enzyme complex might have undergone evolutionary changes in different eukaryotic lineages to adapt according to their lifestyle. The genes encoding the different SnRK1 subunits show considerable differences in copy numbers in the plant lineage, indicating their possible subfunctionalization and specialization (Jansheer K et al., 2019). Further, along with the typical β subunits with both a carbohydrate-binding module (CBM) and a β-C-terminal domain (βCTD), plants also possess shorter β subunits without a CBM (Fig. 1B) (Gissot et al., 2004). The shorter β subunits are also involved in the SnRK1 signaling (Emanuelle et al., 2015). However, the shorter β subunits lack the conserved N-terminal myristoylation (N-MYR) motif. Myristoylation of β subunits is an important mechanism regulating AMPK/Snf1/SnRK1 activity. Initially, AMPKβ myristoylation was identified as an inhibitory mechanism as the disruption of the myristoylation motif of AMPKβ1 enhanced enzyme activity and altered the subcellular localization (Warden et al., 2001). Later, a more complex role for myristoylation in the regulation of AMPK activity under different energy conditions was identified. In energy sufficiency, myristoylation down-regulates AMPK activity by suppressing Thr172 phosphorylation. Energy depletion triggers a myristoyl switch which promotes the membrane association and Thr172 phosphorylation of AMPK (Oakhill et al., 2010). In budding yeast, β subunit myristoylation negatively regulates Snf1 activity by promoting the sequestration of the γ subunit to the plasma membrane (Lin et al., 2003). In Arabidopsis, the loss of N-myristoyltransferase 1 (NMT1) enhanced the endogenous SnRK1 activity. Further, disruption of the myristoylation motif of SnRK1β1 and β2 subunits led to their relocation from the plasma membrane to the nucleus and cytosol, respectively (Pierre et al., 2007). In metabolic stress conditions such as extended night, and photosynthesis inhibitor and hypoxia treatments, SnRK1α is translocated to the nucleus which is important for regulating gene expression. Myristoylation of SnRK1β2 was found to negatively regulate the nuclear translocation of SnRK1α (Ramon et al., 2019).

Plants possess atypical γ subunits, which have possibly originated in green algae (Ramon et al., 2013). Along with the four CBS domains, γ subunits of green plants contain an N-terminal CBM usually found in β subunits (Fig. 1C). These atypical γ subunits in plants are named βγ. The Arabidopsis βγ subunit complements the yeast γ subunit mutant (Ramon et al., 2013). Further functional analysis revealed that the βγ subunit contributes to the SnRK1 complex formation and regulation of gene expression (Ramon et al., 2013; Emanuelle et al., 2015). Thus, in plants, both SnRK1β and βγ subunits possess a CBM; however, the precise function of this domain is yet to be identified. The CBM of AMPKβ subunits binds to glycogen in vitro (Polekhina et al., 2003; McBride et al., 2009; Koay et al., 2010). Glycogen is the major storage form of carbohydrates in animals, and this association sequesters AMPK and inhibits its activity allosterically and by preventing the phosphorylation by upstream kinases (McBride et al., 2009). Thus, AMPK activity is also regulated by the status of the carbohydrate reserves in mammals. In plants, starch is the major storage carbohydrate, and conflicting results reported the binding of SnRK1 CBMs with starch. In in vitro binding assays, SnRK1β2 and βγ subunits showed binding to starch (Avila-Castañeda et al., 2014). However, only SnRK1βγ showed strong binding when a mixture of amylose and amylopectin (starch is a mixture of amylose and amylopectin) was used. Further, starch, but not an amylose and the amylopectin mixture, significantly inhibited SnRK1 activity in the Arabidopsis leaf protein extracts (Avila-Castañeda et al., 2014). However, a later study, using AMPKβ subunits as positive controls, reported that SnRK1 CBMs do not bind to starch and amylose in vitro (Emanuelle et al., 2015). A recent study reported that maltose, a disaccharide produced during starch degradation, binds to SnRK1β1, SnRK1β2 subunits, and the SnRK1βγ/β3 complex in vitro. Further, binding of maltose specifically promoted the activity of the SnRK1α1/βγ/β3 isoenzyme complex at dusk (Ruiz-Gayosso et al., 2018).
Thus, SnRK1 seems to bind to carbohydrates and its activity is possibly connected to starch metabolism in plants. However, more studies are required to identify the molecular details and physiological relevance of this connection.

The γ subunits of AMPK are involved in the regulation of AMPK activity according to the cellular sugar/energy starvation. Adenine nucleotides (ATP, ADP, or AMP) competitively bind to the binding pockets of specific cystathionine-beta-synthase (CBS) domains of the γ subunit (Xiao et al., 2007, 2011; Mayer et al., 2011). This adenylyl charge-dependent regulatory mechanism allows the regulation of AMPK activity according to the extent of sugar/energy starvation (Oakhill et al., 2012). However, in comparison with AMPK, the residues critical for binding of adenine nucleotides are not conserved in plant βγ subunits, which explains the insensitivity of SnRK1 to AMP and ADP treatments (Emanuelle et al., 2015, 2016). Homology modeling revealed that the adenylyl charge-dependent regulatory mechanism that controls the switching of the cellular AMPK pool from inactive to active states, and vice versa, appears to be absent in SnRK1 (Broeckx et al., 2016). Nonetheless, exogenous sucrose (sugar and glucose) treatments altered the expression of marker genes such as ASPARAGINE SYNTHASE 1 (DARK INDUCIBLE 6 (ASN1/DIN6) in an SnRK1-dependent manner (Baena-González et al., 2007; Jamsheer K et al., 2018a). Thus, similar to AMPK signaling, sugar availability is an important regulator of SnRK1 signaling. Recently, trehalose 6-phosphate (T6P) which is produced in low amounts (µM range) is emerging as a major signaling molecule regulating SnRK1 signaling in plants. The T6P level is positively correlated with sucrose availability in Arabidopsis (Lunn et al., 2006). Further, T6P strongly inhibited the plant SnRK1 activity in extracts from diverse plants such as Arabidopsis, broccoli, and spinach. Interestingly, T6P showed no effect on the AMPK/SnF1 activity in extracts from yeast, house fly, or sheep (Zhang et al., 2009). Recent studies suggest an important role of T6P signaling in modulating the SnRK1 signaling network under different environmental conditions in plants (Frank et al., 2018; Zhai et al., 2018; Hwang et al., 2019; for more details, see below). However, as most of the evidence is based on in vitro binding experiments and external feeding of T6P, more studies are needed to clarify the relevance of the T6P pathway in SnRK1 signaling in vivo (for a more elaborate discussion on the interaction between T6P and SnRK1 signaling pathways, please see Figueroa and Lunn, 2016; Baena-González and Lunn, 2020)

Homology modeling suggests that SnRK1 is constitutively active, and therefore protein turnover could be another important regulatory mechanism of SnRK1 signaling in plants (Broeckx et al., 2016). In line with this, negative regulators of SnRK1 such as arginine/serine-rich 45 (SR45) and FCS-like zinc finger 6/10 (FLZ6/10) were found to regulate SnRK1 signaling by affecting the stability of the major kinase subunit SnRK1α1 in Arabidopsis (Carvalho et al., 2016; Jamsheer K et al., 2018a). The regulation of protein stability of the α kinase subunit seems to be dependent on SnRK1 activity as SnRK1α1 mutant proteins (SnRK1α1-T175A and SnRK1α1-K48M) lacking kinase activity showed enhanced accumulation in the Arabidopsis mesophyll protoplast expression system (Baena-González et al., 2007). Ubiquitination and SUMOylation are two important post-translational modifications controlling protein activity and stability. SnRK1 signaling is highly regulated by these modifications in plants (Ananieva et al., 2008; Lee et al., 2008; Carvalho et al., 2016; Crozet et al., 2016). SnRK1α subunits interact with core and accessory components of SCF E3 ligase such as S phase kinase-associated protein 1 (SKP1), and pleiotropic regulatory locus 1 (PRL1), and this complex facilitates the degradation of the kinase (Lee et al., 2008). The SnRK1α subunit was found to interact with SUMO ligase SUMO conjugation enzyme 1 (SCE1) and SUMO proteins SUMO1 and SUMO3 in a high-throughput protein–protein interaction (PPI) screen for identifying SUMO substrates in Arabidopsis (Elrouby and Coupland, 2010). Later, SnRK1α and β subunits were found to be SUMOylated by the SUMO E3 ligase SIZ1, which triggers their ubiquitination and subsequent degradation. This degradation was found to be dependent on the SnRK1 activity, indicating that SUMOylation works as a feedback negative regulatory mechanism of SnRK1 signaling in Arabidopsis (Crozet et al., 2016). Collectively, these results suggest an important regulatory role for ubiquitination and SUMOylation in SnRK1 signaling.

The green plants (Viridiplantae) possess the archetypal α subunits with an N-terminal CD, middle ubiquitin-associated (UBA) domain, and a C-terminal α-CTD, which is important for the interaction with β and βγ subunits (Fig. 1A). It is reported that the UBA domain of AMPKα and Snf1 works as an autoinhibitory domain (AID) (Crute et al., 1998; Jiao et al., 2015). Conversely, the UBA domain of SnRK1α was found to be important for maintaining the catalytic activity in Arabidopsis (Emanuelle et al., 2018). Strikingly, other members of Archaeplastida such as Chondrus crispus (Rhodophyta), Cyanophora paradoxa (Glaucophyta), and other eukaryotic supergroups such as Trypanosoma cruzi (Excavata), Dictostelium purpureum (Amoebozoa), and Entocarpos siliculosus (SAR) do not possess a typical UBA domain signature (Fig. 1A, D). It appears that the UBA domain of α subunits shows high sequence divergence, which could be the reason for the contrasting roles of this domain in different eukaryotic lineages. Collectively, these studies indicate that in comparison with AMPK/Snf1, SnRK1 shows divergence in subunit composition, structure, and regulatory mechanisms, and these differences resulted in a distinct SnRK1 signaling mechanism in the plant lineage (for a more elaborate discussion on SnRK1 structure and regulation, please see Broeckx et al., 2016).

SnRK1 is involved in regulating all aspects of plant growth from seed germination to senescence (Baena-González...
et al., 2007; Jossier et al., 2009; Tsai and Gazzarrini, 2012; Baena-González and Hanson, 2017). In-depth functional analysis of SnRK1 recognized its intricate role in coordinating plant growth according to the environment. PPI and phosphoproteomic analyses revealed its role as a hub protein, communicating with a diverse array of proteins (Arabidopsis Interactome Mapping Consortium, 2011; Cho et al., 2016; Nukarin et al., 2016; Carianopol et al., 2020). However, the biological significance of only a few of these interactions is understood as yet. Compilation of PPI data from the literature and databases revealed that SnRK1 subunits interact with >400 proteins in Arabidopsis (Fig. 2; Table S1 available at the Dryad Digital Repository https://doi.org/10.5061/dryad.c2fqz6178; (Jamsheer K et al., 2021). Most of these interactions

![Fig. 2. The Arabidopsis SnRK1 signaling network based on direct protein–protein interaction and phosphorylation. (A) Interaction and phosphorylation network of Arabidopsis SnRK1 subunits. (B) Interaction subnetwork of SnRK1 subunits and FLZ proteins, highlighting the common interacting proteins. The interaction and phosphorylation data of two SnRK1 α, three β, one βγ subunit, and 18 FLZ proteins of Arabidopsis were retrieved from protein–protein interaction databases (BioGRID v3.5.185, STRING v11.0, IntAct v4.2.14, and AV v2.0) and literature mining. The interactors were annotated using TAIR v10, UniProt v2020_05, and domain analysis using PFAM v32.0. The network was visualized by Cytoscape v3.8.0. Color keys were used to differentiate different functional categories of interactors and to differentiate interaction and phosphorylation. Please refer to Table S1 available at Dryad for more details.](https://academic.oup.com/jxb/advance-article/doi/10.1093/jxb/erab079/6162452)
were identified through yeast two-hybrid (Y2H) screening. Therefore, additional experiments will be needed to verify these interactions in planta and to identify the relevance of these interactions. Nonetheless, these results indicate the role of SnRK1 as a master regulator of plant growth. In line with this, two independent phosphoproteomic studies identified that alterations in SnRK1 signaling affect the phosphorylation states of a large number of proteins in Arabidopsis (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukarinen et al., 2016). SnRK1 is critical for submergence tolerance in plants, and Cho et al. (2016) used a dominant-negative (snrk1a1ΔK48ΔK) mutant of SnRK1α1 to identify the protein phosphorylation sites regulated by the SnRK1 signaling network during submergence at the seedling stage (Fig. 3A). Nukarinen et al. (2016) used the snrk1a1Δ and SnRK1α1 overexpression lines to identify the phosphorylation sites regulated by the SnRK1 signaling network in response to extended night treatment at the rosette stage (Fig. 3D, E). Further, they also developed an inducible artificial miRNA line targeting SnRK1α2 in the background of an snrk1a1 knockout mutant. This snrk1a1α1/α2 line was used to identify phosphorylation sites regulated by the SnRK1 signaling network in the middle and end of the light cycle and in response to extended night treatment at the rosette stage (Fig. 3B, C, F) (Nukarinen et al., 2016). Compilation of these phosphoproteome data revealed that the SnRK1 signaling network regulates the phosphorylation states of >500 proteins in Arabidopsis (Fig. 3; Table S2 at Dryad). Further, PPI analysis in other plants (such as rice and wild soybean) also revealed several interacting proteins of SnRK1 (Fig. 4; Table S1 at Dryad) (Ding et al., 2009; Song et al., 2019).

In this review, we analyzed the SnRK1 interactome from different plants, and phosphoproteomic data from Arabidopsis, to develop an integrative SnRK1 signaling network (Tables S1 and S2 at Dryad). In the following sections, we provide a comprehensive review of the SnRK1 signaling network and their classification based on their molecular functions [such as transcription factors (TFs), metabolic enzymes, and protein kinases]. This network highlights SnRK1 as a multifaceted hub controlling the growth and developmental plasticity of plants according to the environmental conditions. Our analysis also revealed potential novel and uncharted areas of SnRK1 signaling, which are discussed in this review along with their potential biological roles in plants.

**Transcription factors and cofactors**

Transcriptome analyses revealed that perturbation in SnRK1 signaling alters the expression of a large set of genes in Arabidopsis (Baena-González et al., 2007; Pedrotti et al., 2018). Transient overexpression of SnRK1α1 in mesophyll protoplast from rosette leaves resulted in the differential expression of 1021 genes (Baena-González et al., 2007). Transcriptome analysis of rosette leaves of snrk1α1/α2 growing under short-term (6 h) extended darkness revealed 3464 differentially regulated genes in comparison with the wild type (Pedrotti et al., 2018). These datasets showed strong overlap and identified that perturbation in SnRK1 signaling greatly affects the genes involved in primary metabolism (such as carbohydrate, amino acid, and lipid), translation, photosynthesis, and phytohormone and stress signaling machinery in Arabidopsis (Baena-González et al., 2007; Pedrotti et al., 2018). In line with its important role as a global regulator of gene expression, SnRK1 was found to interact with a large number of TFs in Arabidopsis (Fig. 2A; Table S1 at Dryad). The best-studied example of SnRK1-mediated transcriptional regulation under sugar starvation is through TF basic leucine zipper 63 (bZIP63) which belongs to the C-group of bZIPs (Table 1). SnRK1 phosphorylates bZIP63 at specific serine residues during sugar starvation in vivo, which promotes its heterodimerization with S1-bZIPs such as bZIP1 and bZIP11 (Mair et al., 2015). SnRK1α1, bZIP63, and bZIP2 seem to form a ternary complex as the co-expression of each of these proteins enhances the interaction between other proteins in three-hybrid interaction experiments in protoplasts. Further, during starvation, SnRK1 is recruited to the promoter of electron-transfer flavoprotein: ubiquinone oxidoreductase (ETFQO), to promote histone acetylation in a bZIP-dependent manner (Pedrotti et al., 2018). The transcript level of ETFQO is strongly induced in sugar starvation and dark treatments, and it works in the branched-chain amino acid (BCAA) catabolism pathway during dark-induced senescence and sugar starvation. tfp lines show enhanced susceptibility to extended dark treatments, suggesting the crucial role of this mitochondrial enzyme in the survival of plants during sugar starvation (Ishizaki et al., 2005; Pedrotti et al., 2018). Further, RNA-seq analysis revealed that SnRK1 and S1-bZIPs regulate the expression of a large set of genes involved in amino acid catabolism. Thus, the SnRK1–bZIP complex is crucial in inducing the expression of genes important in promoting survival during sugar starvation (Pedrotti et al., 2018).

Recently, the role of the SnRK1 signaling network in regulating the circadian clock is emerging, and bZIP63 plays a crucial role in it. Sucrose shortens the circadian period by repressing the transcription of the circadian oscillator Pseudo-Response Regulator 7 (PRR7) in the late stages of photoperiod (Haydon et al., 2013). A recent study showed that when overexpressed, bZIP63 binds to a specific G-box region of the PRR7 promoter. Gene expression assays using mutant lines identified that bZIP63 up-regulates the expression of PRR7 in low-light conditions. Subsequent analysis using mutants of bZIP63, trehalose phosphate synthase 1 (TPS1), and overexpression lines of SnRK1α1 suggest a role for the T6P–SnRK1–bZIP63 signaling axis in adjusting the circadian phase according to light and dark cycles (Frank et al., 2018). However, most of the experiments were performed with the external feeding of sucrose. Therefore, more studies will be needed for validation of this interesting working hypothesis in natural conditions. Further, the role of SnRK1 was deciphered...
Fig. 3. Compiled network of proteins with altered phosphorylation states due to the perturbation in SnRK1 signaling in Arabidopsis. The phosphoproteomic data of Arabidopsis SnRK1 were retrieved from two studies (Cho et al., 2016; Nukarinen et al., 2016). A dominant-negative mutant of SnRK1α1 (snrk1α1K48M) was used to identify the alteration in the global protein phosphorylation states due to the down-regulation of SnRK1 signaling during submergence stress (Cho et al., 2016). An snrk1α1/α2 line was used to identify the alteration in the global protein phosphorylation states due to the down-regulation of SnRK1 signaling at the middle and end of the light cycle. The snrk1α1, snrk1α1/α2, and SnRK1α1 overexpression lines were used to identify the alteration in the global protein phosphorylation states due to the down-regulation of SnRK1 signaling in response to extended night treatment (Nukarinen et al., 2016). (A) The network of proteins with altered phosphorylation states in snrk1α1K48M during submergence stress. (B and C) The network of proteins with altered phosphorylation states in snrk1α1/α2 at the middle and end of light cycle, respectively. (D) The network of proteins with altered phosphorylation state due to overexpression of SnRK1α1 in extended night treatment. (E) The network of proteins with altered phosphorylation state in snrk1α1 in extended night treatment. (F) The network of proteins with altered phosphorylation state in snrk1α1/α2 in extended night treatment. The target proteins were annotated using TAIR v10, UniProt v2020_05, and domain analysis using PFAM v32.0. The networks were visualized by Cytoscape v3.8.0. Color keys were used to differentiate different functional categories of proteins. Please refer to Table S2 available at Dryad for more details.
using lines overexpressing SnRK1α1. It was previously shown that overexpression of SnRK1α1 lengthens the clock period in light conditions in a time for coffee (TIC)-dependent manner (Shin et al., 2017). Therefore, additional experiments will be needed to verify the role of the SnRK1 signaling network in adjusting the circadian clock according to the photoperiod in plants.

SnRK1 subunits are also reported to be interacting with bZIPS involved in abscisic acid (ABA) signaling such as ABA insensitive 5 (ABI5) and ABRE-binding factor 3 (ABF3) in Y2H assays (Carianopol et al., 2020). Further, some of the TFs involved in ABA signaling, such as ABI5, bZIP12, and ABF2, are phosphorylated by SnRK1 in vitro (Zhang et al., 2008; Bitrián et al., 2011). However, the biological significance of these phosphorylations is yet to be identified. Phosphoproteomic analysis revealed that the down-regulation of SnRK1 signaling leads to a reduction in the phosphorylation states of basic helix–loop–helix (bHLH) TFs such as bHLH122 and bHLH128 involved in ABA signaling during submergence stress in Arabidopsis (Fig. 3A; Table S2 at Dryad) (Cho et al., 2016). Collectively, these results suggest a role for SnRK1 in the ABA signal transduction pathway. SnRK2s works as a major downstream kinase of the ABA signaling pathway in plants (Sun et al., 2019). SnRK2s originate from SnRK1α subunits in the plant lineage (Halford and Hey, 2009; Coello et al., 2011; Jamsheer K et al., 2019). SnRK2-mediated phosphorylation of ABF TFs (ABF1, 2, 3, and 4) is critical for the activation of ABA-dependent transcription in plants (Wang et al., 2013). Thus, along with SnRK2s, SnRK1 seems to be involved in the modulation of these TFs through phosphorylation to promote ABA-mediated regulation of gene expression in a synergistic manner (Rodrigues et al., 2013).

PPI analyses revealed that SnRK1 subunits interact with multiple members of APetala 2 (AP2), Teosinte branched1/Cincinnati/proliferating cell factor (TCP), tandem zinc finger (TZF), MYB, NAC, bHLH, etc. in Y2H assays (Figs 2A, 4; Table S1 at Dryad). However, the biological significance of only a few interactions is well understood. Arabidopsis SnRK1 interacts and phosphorylates Wrinkled 1 (WRI1) in vitro. WRI1 is an AP2 TF involved in the regulation of fatty acid synthesis in seeds, leading to the degradation of WR11 (Table 1). Through this regulation, lipid biosynthesis is negatively regulated in
sugar-limited conditions in plants (Zhai et al., 2017a). Lipid biosynthesis is enhanced in sugar-rich conditions through the suppression of SnRK1, and a pivotal role for T6P was identified in this regulation (Zhai et al., 2017b, 2018). T6P weakened the interaction of SnRK1α1 with the activating kinase SnRK1α1 with NAC TFs identified in Y2H screening (Fig. 2A). A similar signaling network of SnRK1 with NAC TFs can also be speculated on based on the widespread interaction of SnRK1 with NAC TFs identified in Y2H screening (Fig. 2A) (Carianopol et al., 2020). In line with this hypothesis, the NAC TF Suppressors of gamma response 1 (SOG1) was found to be phosphorylated by SnRK1 in low cellular ATP conditions. This phosphorylation is proposed to activate SOG1, which regulates cell cycle activity under low amounts of cellular ATP (Hamasaki et al., 2019).

An increase in ambient temperature results in architectural changes in temperate plants such as Arabidopsis. The bHLH TF phytochrome-interacting factor 4 (PIF4) works as a master TF of the genes involved in hypocotyl elongation in response to temperature increase and reduction in light quality. However, sugar/energy is required for driving this rapid elongation.

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### Table 1. Major characterized phosphorylation targets of SnRK1 and the associated pathway in plants

<table>
<thead>
<tr>
<th>Interactor category</th>
<th>Interactor</th>
<th>Outcome of phosphorylation</th>
<th>Pathway</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcription factor</td>
<td>bZIP63 (Arabidopsis)</td>
<td>Enhanced the heterodimerization with S1-bZIPs in planta</td>
<td>Transcription of sugar starvation-responsive genes</td>
<td>Mair et al. (2015)</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>WR1 (Arabidopsis)</td>
<td>Enhanced the degradation of WR1 in cell-free degradation assay</td>
<td>Seed fatty acid biosynthesis</td>
<td>Zhai et al. (2017a, 2018)</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>PIF4 (Arabidopsis)</td>
<td>Possibly promotes degradation as co-expression of SnRK1α1</td>
<td>Thermomorphogenesis</td>
<td>Hwang et al. (2019)</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>FUS3 (Arabidopsis)</td>
<td>Possibly promotes stability as incubation of protein extract from 3SS:SnRK1α1-HA seedlings enhanced the stability of FUS3</td>
<td>Developmental phase transitions</td>
<td>Tsai and Gazzarrini (2012); Chan et al. (2017)</td>
</tr>
<tr>
<td>Protein synthesis machinery</td>
<td>elF4E (Arabidopsis)</td>
<td>Possibly down-regulates the activity as co-expression of SnRK1α2-CD inhibited the ability of elF4E/elFiso4E to complement elF4E budding yeast mutant in growth assay and polysome formation</td>
<td>Seed development</td>
<td>Bruns et al. (2019)</td>
</tr>
<tr>
<td>Protein phosphatase</td>
<td>PTP1 (Arabidopsis)</td>
<td>Reduced the interaction with MPK6 in planta</td>
<td>MPK6 signaling during submergence</td>
<td>Cho et al. (2016)</td>
</tr>
<tr>
<td>CDK inhibitor</td>
<td>KRP6 (Arabidopsis)</td>
<td>Possibly down-regulates the activity as phosphomimetic KRP6 (KRP6T152D) showed reduced interaction with CycD3:1 in Y2H assay</td>
<td>Cell cycle</td>
<td>Guéritier et al. (2013)</td>
</tr>
<tr>
<td>CDK inhibitor</td>
<td>KRP7 (Arabidopsis)</td>
<td>Possibly down-regulates the activity as phosphomimetic KRP6 (KRP6T152D) showed reduced interaction with CycD3:1 in Y2H assay</td>
<td>Cell cycle</td>
<td>Guéritier et al. (2013)</td>
</tr>
<tr>
<td>Carbohydrate metabolism</td>
<td>SPS (Spinach)</td>
<td>Reduced the in vitro enzyme activity</td>
<td>Sucrose biosynthesis</td>
<td>Sugden et al. (1999)</td>
</tr>
<tr>
<td>Nitrogen metabolism</td>
<td>NR (Spinach)</td>
<td>Reduced the in vitro enzyme activity</td>
<td>Nitrate assimilation</td>
<td>Sugden et al. (1999)</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>DGAT1 (Brassica)</td>
<td>Reduced the in vitro enzyme activity</td>
<td>Triacylglycerol biosynthesis</td>
<td>Caldo et al. (2018)</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>HMG3 (Spinach, Arabidopsis)</td>
<td>Reduced the in vitro enzyme activity</td>
<td>Sterol and isoprenoid biosynthesis</td>
<td>Sugden et al. (1999); Robertlee et al. (2017)</td>
</tr>
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growth. In sugar starvation, SnRK1 phosphorylates PIF4, leading to its degradation via the 26S proteasome (Fig. 2A; Table 1). T6P reduced the phosphorylation of SnRK1α1 by SnRK2 and the phosphorylation of PIF4 by SnRK1α1 in vitro. In line with this, mutants of TPS1 showed reduced PIF4 accumulation and thermoresponsive hypocotyl elongation in Arabidopsis. These results indicate that the T6P–SnRK1–PIF4 signaling axis integrates endogenous sugar status with temperature-mediated alteration of growth in plants (Hwang et al., 2019).

SnRK1 phosphorylates the bHLH TF MYC2 in vitro and promotes its degradation. MYC2 works as the master regulator of jasmonic acid (JA), light, and stress signaling in Arabidopsis. The SnRK1-mediated down-regulation of MYC2 activity attenuates MYC2-dependent salt tolerance (Im et al., 2014). Strikingly, SnRK1 was found to be a positive regulator of JA signaling and JA-mediated defense responses in plants (Hulsmans et al., 2016; Filipe et al., 2018). Further, SnRK1 promotes the degradation of JAZ18, a negative regulator of MYC2 in apple through phosphorylation to promote anthocyanin accumulation (Fig. 4) (Liu et al., 2017). Interaction of Arabidopsis SnRK1 with JAZ3 and JAZ12 is reported in Y2H screens (Arabidopsis Interactome Mapping Consortium, 2011; Carianopol et al., 2020); however, the biological significance of these interactions is not yet known. Nonetheless, these results indicate that SnRK1 might be involved in the regulation of JA signaling.

SnRK1 regulates plant development through phosphorylation-mediated stabilization of the B3 type TF FUSCA3 (FUS3) in Arabidopsis (Fig. 2A; Table 1). This module was found to be important in regulating embryogenesis, developmental phase transitions, flowering, lateral organ and seed development, and heat stress responses (Tsai and Gazzarrini, 2012; Chan et al., 2017). The C2H2 type TF IDD8 is a target of SnRK1 to delay flowering in Arabidopsis during sugar starvation. SnRK1 phosphorylates IDD8 in the nucleus, which inhibits its TF activity, leading to a delay in flowering (Jeong et al., 2015). In phosphoproteomics analysis, phosphorylation states of other IDD family TFs (IDD1, IDD5, and IDD6) were found to be down-regulated in snk1α1/α2 or snk1α1 K48M lines under extended night or submergence stress treatments (Fig. 3A, F) (Nukarinen et al., 2016). Thus, the IDD TF family could be an important target of SnRK1 signaling in Arabidopsis. Yeast three-hybrid (Y3H) and co-immunoprecipitation (Co-IP) assays revealed that rice SnRK1α1 and Heading date repressor 1 (HDR1) form a complex with the B-box TF Heading date 1 (HD1), which leads to the phosphorylation of HD1 (Fig. 4). HD1 is the homolog of Arabidopsis Constans (CO), the master regulator of photoperiodic control of flowering. From the genetic analysis, the SnRK1–HDR1–HD1 complex was found to be crucial in repressing flowering in rice (Sun et al., 2016).

SnRK1 negatively regulates senescence in plants (Baena-González et al., 2007). This is partly achieved through the suppression of ethylene signaling, the promoter of senescence in plants. SnRK1 phosphorylates ethylene insensitive 3 (EIN3), a key TF in ethylene signaling in vitro. In the protoplast assays, overexpression of catalytically active SnRK1α1 negatively regulated the stability of EIN3, suggesting that the SnRK1-mediated phosphorylation negatively regulates EIN3 activity (Fig. 2A) (Kim et al., 2017). Y2H screening revealed that SnRK1 subunits from Arabidopsis, rice, and tomato interact with multiple members of the MYB class of TFs (Figs 2A, 4; Table S1 at Dryad). Similarly, the interaction of SnRK1 subunits with several members of ZF-HD, TCP, TZF, GATA, HD-ZIP, GeBP, DBB, and WRKY TF families is identified in the interaction screening experiments (Figs 2A, 4; Table S1 at Dryad). It could be possible that SnRK1 phosphorylates these TFs to control plant growth in different environmental conditions. In line with this, phosphoproteomic analysis in Arabidopsis identified that the down-regulation of SnRK1 signaling changes the phosphorylation state of several members of TCP, TZF, HD-ZIP, GeBP, WRKY, and MYB TF families under different time points of the light cycle, extended night, or submergence stress treatments (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukarinen et al., 2016). More studies focused on the interaction of specific TFs with SnRK1 are required to identify the biological significance of these connections.

Apart from direct interaction with TFs, SnRK1 also interacts with regulators of TFs, especially those involved in phytohormone signaling (Figs 2A, 4; Table S1 at Dryad). Further, alteration in SnRK1 signaling affected the phosphorylation states of many key transcriptional regulators under different treatment conditions (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukarinen et al., 2016). As described previously, SnRK1 interacts with JAZ proteins in Arabidopsis and apple, and promotes phosphorylation-mediated degradation of JAZ18 in apple (Arabidopsis Interactome Mapping Consortium, 2011; Liu et al., 2017; Carianopol et al., 2020). In Y2H assays, the interaction of Arabidopsis SnRK1α1 with the DELLA protein RGA-LIKE3 (RGL3), which works as a positive regulator of JA and a negative regulator of gibberellin (GA) signaling, was identified (Wild et al., 2012; Carianopol et al., 2020). Similarly, Y2H assays revealed the interaction of SnRK1 with the NINJA protein AB15-binding protein 2 (AFP2) and the transcriptional regulator Dynamic influencer of gene expression 1 (DIG1), which are negative regulators of ABA signaling in Arabidopsis (Song et al., 2016; Chang et al., 2018; Carianopol et al., 2020). SnRK1 signaling is highly integrated into phytohormone signaling networks in plants (Jamsheer K et al., 2019). Identification of the biological significance of these interactions will be crucial in identifying the important molecular links of SnRK1–phytohormone signaling interactions in plants.

In conclusion, multiple TFs and cofactors could serve as downstream signaling partners of SnRK1 in regulating plant development through interacting with phytohormone, nutrient, and stress signaling pathways to coordinate gene expression in different environmental conditions.
Chromatin remodelers and epigenetic modifiers

The PPI and phosphoproteomics analyses revealed the association of SnRK1 with core transcriptional machinery, chromatin remodeling, and epigenetic control of gene expression (Figs 2A, 3; Tables S1, S2 at Dryad). The soybean SnRK1 interacts with histone acetyltransferase 1 (HAT1) in Y2H assays (Fig. 3) (Song et al., 2019). In Arabidopsis, alterations in the SnRK1 signaling altered the phosphorylation states of several key proteins involved in histone acetylation, methylation, and chromatin remodeling during submergence stress or extended night treatments (Fig. 3) (Cho et al., 2016; Nukarinen et al., 2016). This includes proteins involved in histone modification and chromatin remodeling such as histone deacetylase 19 (HD19), Early Flowering 7 (ELF7), PWPP domain protein 2 (PDP2), Modifier of SNC1 (MOS1), and DAYSLEEPER (Fig. 3; Table S2 at Dryad). Whether SnRK1 is directly involved in changing these phosphorylation states is yet to be determined. Nonetheless, these preliminary observations suggest a possible role for SnRK1 in histone modifications and chromatin remodeling in plants. In Opisthokonta, the role of AMPK/Snfl as a kinase regulating the activity of histone modification enzymes and chromatin remodelers is well established (Lo et al., 2001; Bungard et al., 2010; Marin et al., 2017; Gongol et al., 2018). In mammals, AMPK associates with the promoters of glucose and lipid metabolism genes during starvation (Ratman et al., 2016). Similarly, SnRK1 associates with the promoter of ETFQO which is further enhanced in extended dark treatment. This association was found to be dependent on bZIP63 and S1-bZIPs, and the SnRK1–bZIP complex was found to be crucial in inducing the expression of ETFQ by promoting histone 3 lysine 14 (H3K14) acetylation during dark treatment (Pedrotti et al., 2018). Thus, similarly to AMPK/Snfl, SnRK1 seems to form a regulatory complex in the nucleus with TFs, histone modifiers, and chromatin remodelers to regulate gene expression, especially under starvation and stress conditions. However, more studies are needed to establish a direct role for SnRK1 and to identify the key phosphorylation substrates.

Regulators of RNA metabolism and processing

In the phosphoproteomics analyses, alterations in SnRK1 signaling changed the phosphorylation states of a large number of proteins involved in RNA metabolism especially in different stages of the light cycle and extended night conditions (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukarinen et al., 2016). This includes key proteins such as Varicose (VCS), RNA-binding protein 25 (RBM25), STAR1K1 (STA1), EARLY FLOWERING 9 (ELF9), and splicing factor 1 (SF1) (Fig. 3). Thus, the SnRK1 signaling network seems to regulate crucial proteins involved in mRNA and small RNA biogenesis, constitutive and alternative splicing, RNA stability, and catabolism. Delineating the functional significance of these phosphorylations and identifying the direct and/or indirect role of SnRK1 will help in elucidating the mechanistic links connecting nutrient status and RNA metabolism and processing in plants. AMPK is involved in the negative regulation of rRNA biogenesis during energy starvation through phosphorylation-mediated inhibition of the RNA polymerase I-associated TF TIF-IA (Hoppe et al., 2009). Although the mechanistic details are not known, overexpression of SnRK1A1 in mesophyll protoplasts led to down-regulation of the transcript level of a large set of rRNA and rRNA biogenesis genes in Arabidopsis (Baena-González et al., 2007). Thus, SnRK1 seems to be involved in adjusting RNA biogenesis according to the nutrient status in plants. Previously, Arabidopsis SR45, a spliceosome component involved in the regulation of RNA splicing and metabolism, was found to be a negative regulator of SnRK1A1 protein stability (Carvalho et al., 2016), suggesting a reciprocal connection of SnRK1 and RNA metabolism and processing machinery.

Components of the protein synthesis machinery

Protein synthesis is an energy-demanding process (Lindqvist et al., 2018). During starvation, SnRK1 homologs in eukaryotes are known to limit protein synthesis while promoting the synthesis of a subset of proteins involved in metabolic adaptation during energy deficit (Broeckx et al., 2016; Lin and Hardie, 2018). This is achieved through phosphorylation-mediated control of regulators of protein synthesis and repression of rRNA gene expression (Baena-González et al., 2007; Broeckx et al., 2016). Target of rapamycin complex 1 (TORC1) is a promoter of protein synthesis and rRNA expression in nutrient sufficiency conditions (Dobrenel et al., 2016). In mammals, AMPK down-regulates TORC1 activity during energy starvation through the phosphorylation-mediated activation of Tubular sclerosis complex (TSC) and phosphorylation-mediated negative regulation of Regulatory-associated protein of TOR (RAPTOR), a crucial accessory protein of TORC1 (Inoki et al., 2003; Gwinn et al., 2008; Hindupur et al., 2015). Homologs of TSC proteins are absent in the plant lineage. Arabidopsis SnRK1A1 interacted with RAPTOR1B in planta and phosphorylated it in an in vitro kinase assay (Nukarinen et al., 2016). Although it is yet to be demonstrated, SnRK1–mediated phosphorylation of RAPTOR might be important in down-regulating TORC1 activity in plants. In line with this, phosphoproteome analysis revealed that the phosphorylation at Ser240 of ribosomal protein S6A (RPS6A) and RPS6B, the conserved targets of TORC1 signaling to regulate protein synthesis in eukaryotes, was found to be down-regulated by SnRK1 in Arabidopsis (Fig. 3B; Table S2 at Dryad) (Nukarinen et al., 2016). Intriguingly, phosphorylation of another motif in RPS6A was found to be
down-regulated in snrk1α1/α2 under extended night treatment (Fig. 3F) (Nukarinen et al., 2016). This phosphorylation might be relevant in regulating protein synthesis during sugar starvation as studies have shown that RPS6s are one of the most important substrates of phosphorylation in the 40S subunit of the ribosome, and their phosphorylation states were found to be dynamically regulated by phytohormones, light, and various stress conditions in plants (Browning and Bailey-Serres, 2015). Further, phosphoproteomics analyses revealed that alterations in SnRK1 signaling affect the phosphorylation states of core proteins involved in the mRNA translation and ribosome assembly (Fig. 3; Table S2 at Dryad). Identification of the direct and/or indirect role of SnRK1 in the regulation of these phosphorylations may reveal potential TOR-independent mechanisms controlling protein synthesis during sugar starvation or stress conditions in plants.

SnRK1 exerts direct control over protein synthesis by phosphorylating the 5′ cap-binding initiation factors, eukaryotic translation initiation factor 4E (eIF4E) and eukaryotic translation initiation factor isoform 4E (eIFiso4E) (Fig. 2A; Table 1). These phosphorylations inhibit their activity and polysome formation (Brüns et al., 2019). During hypoxia, SnRK1 activity is enhanced, which phosphorylates eukaryotic translation initiation factor isoform 4G1 (eIFiso4G1) and eIFiso4G2 that in turn promote the translation of core hypoxia and stress response genes (Cho et al., 2019).

**Protein kinases and phosphatases**

SnRK1 interacts with several members of different protein kinase and protein phosphatase families (Fig. 2A; Table S1 at Dryad). Functional analyses have revealed that they work both upstream and downstream of SnRK1 in the signaling network (Broeckx et al., 2016). In tomato, AvrPto-dependent Pto-interacting protein 3 (Adi3), a phototropin–like protein kinase, interacts with α kinase and β regulatory subunits of SnRK1 and phosphorylates a specific β subunit in vivo (Fig. 4). This phosphorylation reduces the SnRK1 activity (Avila et al., 2012).

Recent studies show that the SnRK1 signaling network is highly connected to SnRK2 and SnRK3 signaling networks in plants (Jamsheer K et al., 2019). PP2Cs work as negative regulators of all three types of SnRKs (Vlad et al., 2009; Lan et al., 2011; Rodrigues et al., 2013; Singh et al., 2018). Y2H screening identified the interaction of SnRK1 with members of the PP2C family in rice and soybean (Fig. 4; Table S1 at Dryad). An SnRK1–SnRK2–PP2C regulatory complex important in regulating plant growth was identified recently in Arabidopsis (Belda-Palazón et al., 2020). In favorable growth conditions, the subgroup III SnRK2s (SnRK2.2, SnRK2.3, and SnRK2.6) bind and sequester SnRK1α1 to the SnRK2–PP2C complex, leading to the suppression of SnRK1 signaling and promotion of TORC1 signaling and growth. Under stress conditions, ABA signaling promotes the disassembly of this complex, leading to TORC1 inhibition and stress responses in plants (Wang et al., 2018; Belda-Palazón et al., 2020). Co-IP analyses revealed a direct interaction of SnRK1α1 with TOR and RAPTOR1B. Further, these interactions were enhanced by short-term ABA treatment, suggesting that SnRK1 may play an important role in the down-regulation of TORC1 signaling during stress conditions (Belda-Palazón et al., 2020). SnAK2 phosphorylates an SnRK3 kinase, salt overly sensitive 2 (SOS2), in vitro, and this phosphorylation was found to be important in enhancing the activity of SOS2 (Barajas-Lopez et al., 2018). In rice, an SnRK3 named calcineurin B-like-interacting protein kinase 15 (CIPK15) interacts with an SnRK1α subunit in vivo and promotes its level in response to sugar starvation (Lee et al., 2009). In Arabidopsis, the interaction of SnRK1 subunits with multiple members of CIPK/SnRK3 kinases involved in the regulation of stress responses and plant development was identified through Y2H screening (Fig. 1A; Table S1 at Dryad) (Carianopol et al., 2020). The activity of CIPKs is regulated by calcineurin B-like (CBL) proteins, and Arabidopsis SnRKβ1 interacts with CBL1 in planta (Li et al., 2013). These interactions suggest the presence of an SnRK1–CIPK/SnRK3–CBL signaling network controlling stress responses in plants (Fig. 2A). However, further studies are needed to establish the functional hierarchy of this signaling network.

PPI screening revealed that Arabidopsis SnRK1 interacts with mitogen-activated protein kinase 6 (MAPK6; MPK6) and several other uncharacterized protein kinases with the MAPK domain (Fig. 1A; Table S1 at Dryad) (Cho et al., 2016; Carianopol et al., 2020). SnRK1 interacts with both MPK6 and protein tyrosine phosphatase 1 (PTP1) in planta. PTP1 inactivates MPK6 through dephosphorylation. SnRK1 phosphorylates PTP1 in vitro and this phosphorylation disrupted PTP1–MPK6 association. In line with this, further analysis revealed that SnRK1 promotes MPK6 signaling during submergence (Table 1) (Cho et al., 2016). Further studies are needed to identify the biological significance of the potential SnRK1–MAPK signaling cascade in plants.

Arabidopsis SnRK1 interacts with two cyclin-dependent kinases (CDKs), CDKE1 and CDKF1 (Fig. 2A; Table S1 at Dryad) (Arabidopsis Interactome Mapping Consortium, 2011; Ng et al., 2013). CDKE1 interacts with SnRK1 in the nucleus, and this complex is possibly involved in retrograde signaling (Ng et al., 2013). The interaction of SnRK1 with CDKF1 is identified in Y2H screening, and the functional significance of this interaction is not yet known (Arabidopsis Interactome Mapping Consortium, 2011). Further, Arabidopsis SnRK1 phosphorylates CDK inhibitors Kip-related protein 6 (KP6) and KRP7 in vitro (Fig. 2A; Table 1). In KP6, this phosphorylation occurs at the CDK/cyclin binding domain and reduced the binding with CycD3;1 in Y2H assay (Guérinier et al., 2013). AMPK phosphorylates KRP homologs in mammals at specific threonine residues, which leads to cytoplasmic relocalization and enhanced stability (Liang et al., 2007; Short...
et al., 2008). Although the mechanism of AMPK/SnRK1-mediated regulation is different in plants and animals, KRP s appear to be conserved downstream factors involved in the regulation of CDK/cyclin activity and cell cycle control.

The PPI analysis revealed that SnRK1 from different plants interacts with members of several protein kinases families such as receptor-like kinase (RLK), leucine-rich-repeat receptor kinase (LRR–RK), interleukin-1 receptor-associated kinase (IRAK), histidine kinase (HK), and with no lysine (K) (WNK) kinase, in a Y2H system (Figs 2A, 4; Table S1 at Dryad). Similarly, interaction of Arabidopsis SnRK1 subunits with protein phosphatases such as SCP1-like small phosphatase 4 (SSP4) and starch–excess 4 (SEX4) was identified (Fig. 2A; Table S1 at Dryad) (Fordham-Skelton et al., 2002; Carianopol et al., 2020). Further studies are needed to identify the biological significance of these interactions. Nevertheless, these results indicate that protein kinases and phosphatases work both upstream and downstream of SnRK1 signaling.

**Metabolic enzymes**

**Carbohydrate metabolism**

Alteration in SnRK1 signaling affects metabolic adaptations of plants, especially related to the diurnal cycle and sugar starvation. Plants synthesize starch in chloroplasts during the day, which is mobilized to other tissues during the night for providing energy and storage. In Arabidopsis, studies identified that perturbation in SnRK1 signaling impairs starch accumulation and mobilization in source and sink tissues (Baena-González et al., 2007; Nukarinen et al., 2016). In wheat and rice, transient assays identified that SnRK1 is required for driving the expression of α-amylases, a crucial class of enzymes involved in starch mobilization (Laurie et al., 2003; Lin et al., 2014). In moss, double mutants of SnRK1α1 subunits were unable to survive in normal day–night growth conditions and required continuous light. This was found to be due to the reduced ability of the mutant to accumulate starch during the light cycle (Thelandersson et al., 2004). Thus, SnRK1 appears to be a regulator of both starch production and mobilization.

SnRK1, in general, promotes the expression of photosynthesis- and catabolism-related genes, while it suppresses the genes involved in anabolism (Baena-González et al., 2007; Zhang et al., 2009). Further, SnRK1 directly regulates metabolism through phosphorylation-regulated control of enzyme activity (Table 1). In spinach, SnRK1 phosphorylates and inactivates sucrose-phosphate synthase (SPS), a key enzyme in sucrose biosynthesis in *in vitro* (Table 1) (Sugden et al., 1999). The strawberry SnRK1 also interacts with SPS enzymes in Y2H assays, which is a positive regulator of sucrose accumulation in fruits (Luo et al., 2020). In Arabidopsis, overexpression of SnRK1α1 led to the up-regulation of the phosphorylation status of SPS1F and SPS4F (Nukarinen et al. 2016). Taken together, SPSs seem to be a major target of SnRK1 in controlling sucrose synthesis. In addition, SnRK1 phosphorylates fructose-2,6-bisphosphatase (F2KP), another enzyme involved in carbohydrate metabolism in *in vitro* assays (Kulma et al., 2004; Cho et al., 2016). In potato, a vacuolar invertase (INV), named INV1, and its inhibitor, INV inhibitor 2B (INVInh2B), form an invertase-regulation protein complex (IRPC) with SnRK1. The SnRK1β subunit promotes INV1 activity through inhibiting INVInh2B. Intriguingly, the SnRK1α subunit phosphorylates the SnRK1β subunit, leading to enhanced activity of INVInh2B. Thus, the IRPC regulates the sweetening of potato tubers through regulating sucrose hydrolysis to glucose and fructose (Lin et al., 2015). SnRK1 present in endosperm extract negatively regulates the activity of glyceraldehyde-3-phosphate dehydrogenases (GAPDH), a key class of enzyme in glycolysis, by phosphorylation in wheat (Piattomi et al., 2011, 2017). The interaction of SnRK1 with carbonic anhydrase (CA), pyruvate kinase (PK), sucrose synthase (SUS), and other enzymes involved in photosynthesis and carbohydrate metabolism has been reported from different plants (Figs 2A, 4; Table S1 at Dryad) (Song et al., 2019; Carianopol et al., 2020; Luo et al., 2020). Taken together, SnRK1 appears to be a central regulator of carbohydrate metabolism and metabolic adjustment in different environmental conditions in plants. In line with this, the Arabidopsis snrk1α1/α2 mutant showed a significant difference in the level of sugars, sugar alcohols, and tricarboxylic acid cycle intermediates in comparison with the wild type in extended night treatment (Nukarinen et al., 2016).

**Trehalose metabolism**

As discussed previously, the T6P-mediated control of SnRK1 signaling was found to be important in many signaling pathways (Zhai et al., 2018; Hwang et al., 2019). Intriguingly, SnRK1 signaling seems to be reciprocally connected to T6P signaling as the reduction in the T6P levels in the early stages of submergence in Arabidopsis was found to be abolished in the dominant-negative mutant (snrk1α1K48A) of SnRK1α1 (Cho et al., 2016). Further, the interaction of Arabidopsis TPS11 with SnRK1 was identified in Y2H screening (Fig. 2A) (Carianopol et al., 2020). Although TPS11 belongs to the non-enzymatic class II TPSs (Ramon et al., 2009; Delorge et al., 2015), it might play a regulatory role in the T6P biosynthesis pathway. More focused studies are needed to establish the potential reciprocal interaction of T6P and SnRK1 signaling in plants.

**Inositol metabolism**

SnRK1 phosphorylates inositol polyphosphate kinase 2 beta (IPK2β) *in vitro* (Fig. 2A) (Yang et al., 2018). In the yeast complementation assay, co-expression of SnRK1α1, but not SnRK1α1K48A (catalytically inactive mutant), with IPK2β prevented the complementation of the budding yeast ipk2 mutant, indicating that SnRK1-mediated phosphorylation possibly down-regulates the IPK2β activity.
In Arabidopsis, IPK2β works as an inositol polyphosphate multikinase (IPMK) involved in the biosynthesis of phytate (myo-inositol 1,2,3,4,5,6-hexakisphosphate), a storage form of phosphorus involved in the regulation of plant development (Yang et al., 2018). Interestingly, in mammals, IPMK is involved in the regulation of AMPK and mammalian TOR complex 1 (mTORC1) signaling. In high-glucose conditions, activated IPMK binds to AMPK to down-regulate its activity through promoting its phosphorylation (Bang et al., 2012). IPMK interacts with mTORC1 to promote the mTOR and RAPTOR association, thereby working as an activator of mTORC1 signaling in amino acid sufficiency (S. Kim et al., 2011). Thus, IPMKs appear to be reciprocally connected to SnRK1–TORC1 signaling in plants. In Arabidopsis, inositol-polyphosphate 5-phosphatase 13 (5PTase13) interacts with SnRK1 and regulates its activity in a biphasic manner. During low-nutrient conditions, 5PTase13 promotes SnRK1 activity, whereas, in severe starvation, it negatively regulates SnRK1 activity (Ananieva et al., 2008).

Nitrogen, amino acid, and polyamine metabolism

Nitrate reductase (NR), the critical enzyme that catalyzes the first step in nitrate assimilation, was one of the first targets of SnRK1 identified in plants (Figs 2A, 4; Table 1). In spinach, phosphorylation of NR by SnRK1 inhibits its activity in vitro (Sugden et al., 1999). In Arabidopsis, SnRK1 promotes the phosphorylation of NR1 and NR2 to negatively regulate NR activity (Li et al., 2009; Nukarinen et al., 2016). Thus, NRs could be one of the primary targets of SnRK1 to regulate nitrate assimilation in plants. In yeast and mammals, the activity of Snf1/AMPK is tightly regulated according to the cellular nitrogen level. Low nitrogen activates Snf1/AMPK, and amino acid metabolism (Nukarinen et al., 2016). Arabidopsis SnRK1 also interacts with aspartate oxidase (AO), a key enzyme in NAD biosynthesis in Y2H assays (Carianopol et al., 2020). Similarly, high-throughput Y2H screening identified the interaction of rice SnRK1 with 2-isopropylmalate synthase B (IPMSB), a crucial class of enzyme that catalyzes the first step of leucine biosynthesis (De Kraker et al., 2007; Ding et al., 2009). However, the biological significance of these interactions is yet to be identified. Nonetheless, an Arabidopsis SnRK1α double mutant showed alteration in the level of amino acids in extended night period treatment, indicating a role for SnRK1 signaling in amino acid metabolism (Nukarinen et al., 2016).

Polyamines are aliphatic nitrogenous compounds involved in the regulation of plant growth and stress responses. The Arabidopsis SnRK1α double mutant showed enhanced accumulation of polyamines in response to extended night treatment, indicating the role of SnRK1 signaling in polyamine metabolism (Nukarinen et al., 2016). In line with this, Y2H screening identified the interaction of Arabidopsis SnRK1 with S-adenosylmethionine decarboxylase 2 (SAMDC2) and polyamine oxidase 3 (PAO3) (Fig. 2A) (Carianopol et al., 2020). Identification of the biological significance of these interactions will help in identifying the molecular link of SnRK1 signaling with polyamine metabolism in plants.

Lipid metabolism

Sugars provide energy, and work as the carbon skeleton for lipid biosynthesis. At the molecular level, sugars promote lipid biosynthesis by enhancing the level of WR11 (Zhai et al., 2017b). Thus, SnRK1 signaling, which is under the direct control of cellular sugar status and T6P, appears to be a regulatory hub in adjusting lipid biosynthesis according to the sugar availability in plants (Zhai et al., 2017a, 2018). The overexpression and RNAi-mediated suppression of the SnRK1αt1 level led to reduced triacylglycerol (TAG) levels in the seeds of Arabidopsis (Zhai et al., 2017a). At the molecular level, SnRK1-mediated phosphorylation promotes the degradation of WR11, the positive regulator of fatty acid synthesis in seeds (Zhai et al., 2017a, 2018). Rapeseed SnRK1 phosphorylates and inactivates diacylglycerol acyltransferase 1 (DGAT1) in vitro (Fig. 4; Table 1). DGAT1 catalyzes the final biosynthesis of tryptophan, serine, and histidine was found to be altered (Baena-González et al., 2007). In fact, the expression level of ASN1/DIN6, an asparagine synthase gene, is widely used as marker to monitor changes in SnRK1 activity (Baena-González et al., 2007; Rodrigues et al., 2013; Mair et al., 2015; Frank et al., 2018; Jamsheer K et al., 2018a). Further, SnRK1 interacts with enzymes involved in nitrogen and amino acid metabolism (Figs 2A, 4). In the Y2H screening, the interaction of Arabidopsis SnRK1 with enzymes involved in amino acid metabolism such as asparaginase B1 (ASPGB1) and N-acetyltransferase activity 1 (NATA1) was identified (Carianopol et al., 2020). Arabidopsis SnRK1 also interacts with aspartate oxidase (AO), a key enzyme in NAD biosynthesis in Y2H assays (Carianopol et al., 2020). Similarly, high-throughput Y2H screening identified the interaction of rice SnRK1 with 2-isopropylmalate synthase B (IPMSB), a crucial class of enzyme that catalyzes the first step of leucine biosynthesis (De Kraker et al., 2007; Ding et al., 2009). However, the biological significance of these interactions is yet to be identified. Nonetheless, an Arabidopsis SnRK1α double mutant showed alteration in the level of amino acids in extended night period treatment, indicating a role for SnRK1 signaling in amino acid metabolism (Nukarinen et al., 2016).

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step in TAG biosynthesis, and this phosphorylation site was found to be conserved in DGAT homologs in other plants, suggesting DGATs as the target of SnRK1 in diverse plant species (Caldo et al., 2018). Further, SnRK1 phosphorylates and inactivates HMG-CoA reductase (HMGCR) in spinach and Arabidopsis in vitro. HMGCR is a key enzyme in the mevalonate pathway for sterol and isoprenoid biosynthesis in plants (Table 1) (Sugden et al., 1999; Robertlee et al., 2017). Thus, SnRK1 works as a major regulator of lipid biosynthesis in plants. In Arabidopsis, Y2H screening identified the interaction of SnRK1 with monogalactosyldiacylglycerol synthase type C (MGDC) and long-chain acyl-CoA synthetase 8 (LACS8), which are involved in galactolipid and cuticle biosynthesis, respectively (Fig. 2A) (Kobayashi et al., 2009; Zhao et al., 2019; Carianopol et al., 2020). Arabidopsis SnRK1 phosphorylates phospholipid hydrolyses cytidylyltransferase 1 (CCT1) in vitro. This phosphorylation possibly inhibits the CCT1 catalytic activity and in planta phosphatidylcholine biosynthesis (Caldo et al., 2019). Interaction of Arabidopsis SnRK1 with several uncharacterized lipases was identified in Y2H assays (Fig 2A) (Carianopol et al., 2020). Although the significance of these interactions is yet to be established, these results indicate that SnRK1 is a major regulator of lipid metabolism in plants.

Other metabolic pathways

SnRK1 interacts with alcohol dehydrogenase 1 (ADH1), the key ethanol dehydrogenase critical for anaerobic respiration in hypoxia in the Y2H system (Fig. 2A) (Xu et al., 2006; Carianopol et al., 2020). SnRK1 plays a crucial role in submergence tolerance through enhancing the expression of ADH1 and promoting the translation of ADH1 and other hypoxia response genes in Arabidopsis (Cho et al., 2016, 2019). The direct interaction with ADH1 suggests the possible regulation of ADH1 activity by SnRK1 through phosphorylation. SnRK1 is possibly involved in redox regulation as the interaction of SnRK1 with a glutaredoxin, ROXY8, in the Y2H system (Fig. 2A) (Carianopol et al., 2020). Intriguingly, the activity of Arabidopsis SnRK1 was found to be regulated by redox status. In vitro assays identified that the enzyme activity of Arabidopsis SnRK1 is high in reducing conditions. This regulation was found to be dependent on a conserved cysteine residue in the T-loop (Wurzinger et al., 2017). Y2H screening identified the interaction of Arabidopsis SnRK1 with cytochrome P450 enzymes involved in ABA and GA metabolism (Fig. 2A) (Carianopol et al., 2020). Identification of the biological significance of these interactions will reveal the molecular nodes of SnRK1 and metabolic network interaction in plants.

Channels and transporters

Studies on mammalian models identified that AMPK regulates the activity of channels and transporters through phosphorylation (Lang and Föllér, 2014). For example, AMPK phosphorylates Kv2.1, a voltage-gated potassium channel, to reduce membrane excitability in neurons (Ikematsu et al., 2011). In plants, the direct role of SnRK1 in controlling the activity of channels and transporters is yet to be identified. Nonetheless, PPI screens identified the interaction of SnRK1 with many channels and transporters in Y2H assays (Figs 2A, 4; Table S1 at Dryad) (Chen et al., 2012; Carianopol et al., 2020). In Y2H assays, Arabidopsis SnRK1 interacts with KAT1, an inward-rectifier potassium channel that belongs to the Shaker family with important roles in controlling stomatal function (Fig. 2A) (Carianopol et al., 2020). Similarly, rice SnRK1 also interacts with an inward-rectifying potassium channel named potassium transport 2/3 (AKT2/3) (Fig. 4) (Rohila et al., 2009). PPI analyses using Y2H assay revealed the interaction of Arabidopsis SnRK1 with cyclic nucleotide-gated channel 12 (CNGC12), CNGC13, and CNGC18 (Fig. 2A) (Chen et al., 2012; Carianopol et al., 2020). Similarly, Y2H analyses revealed the interaction of SnRK1 with several transporters involved in regulating nutrient uptake, nutrient and ion transport, and homeostasis (Figs 2A, 4; Table S1 at Dryad). SnRK1 interacts with phosphate transporter 1:4 (PHT1;4) and PHO1 homolog 7 (PHO1;7) which are involved in phosphate uptake and loading of phosphate into the xylem vessels in the root, respectively (Carianopol et al., 2020). Soybean SnRK1 interacts with nitrate transporter 2.4 (NT2.4) (Song et al., 2019). Further, SnRK1 interacts with sugar transporter 4 (STP4), sucrose–proton symporter 1 (SUC1), ERD6-like 4 (ERD6-L4), and plastid glucose transporter (PGLCT) in the Y2H system (Chen et al., 2012; Carianopol et al., 2020). In Y2H assays, Arabidopsis SnRK1 also interacts with transporters involved in amino acid and peptide transport such as cationic amino acid transporter 6 (CAT6), glutamine dumper 2 (GDU2), GDU4, and peptide transporter 2 (PTR2) (Chen et al., 2012). Further studies will be needed to verify these interactions in planta and to identify the biological significance of these interactions. It could be possible that through regulating the activity of these channels and transporters, SnRK1 might be involved in nutrient uptake and transport, amino acid and sugar transport, stomatal functions, etc. In line with this, changes in SnRK1 signaling altered the phosphorylation states of many crucial transporters involved in sugar, ion, and water transport in Arabidopsis (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukeninen et al., 2016). More targeted studies are needed to identify the direct/in-direct role of SnRK1 in controlling the activity of channels and transporters in plants.

Cytoskeleton, organelle movement, and membrane vesicle trafficking

The cytoskeleton is a highly regulated and dynamic network of protein filaments in cells that play a crucial role in organelle movement and vesicle trafficking (Rogers and Gelfand, 2011). It consists of three main types of filaments: actin, microtubules, and intermediate filaments. Actin filaments are primarily involved in cell motility and endocytosis, while microtubules are responsible for intracellular transport and vesicle trafficking. Intermediate filaments provide structural support and mechanical strength to the cellular matrix. SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) are proteins that facilitate the membrane fusion of vesicles and their interactions with the cytoskeleton. They play a crucial role in the transport of vesicles throughout the cytoplasm. Mutations in SNARE genes can lead to defects in vesicle transport and cellular homeostasis, highlighting their importance in maintaining cellular function. In plants, SnRK1 interacts with several transporters involved in nutrient uptake, nutrient and ion transport, and homeostasis (Figs 2A, 4; Table S1 at Dryad). The interaction of SnRK1 with transporters such as potassium transporters, phosphate transporters, and sugar transporters is consistent with the role of SnRK1 in controlling nutrient uptake and loading. This suggests that SnRK1 might be involved in nutrient uptake and transport, amino acid and sugar transport, stomatal functions, etc. Further studies are needed to verify these interactions in planta and to identify the biological significance of these interactions. It could be possible that through regulating the activity of these channels and transporters, SnRK1 might be involved in nutrient uptake and transport, amino acid and sugar transport, stomatal functions, etc. In line with this, changes in SnRK1 signaling altered the phosphorylation states of many crucial transporters involved in sugar, ion, and water transport in Arabidopsis (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukeninen et al., 2016). More targeted studies are needed to identify the direct/in-direct role of SnRK1 in controlling the activity of channels and transporters in plants.
J-domain protein 2A (ERDJ2A), an integral endoplasmic reticulum membrane protein with possible function in protein translocation (Yamamoto et al., 2008; Lee et al., 2011; Chen et al., 2012; Carianopol et al., 2020). Phosphoproteome analysis revealed that perturbation in SnRK1 signaling affects the phosphorylation states of several key proteins involved in protein trafficking (Cho et al., 2016; Nukarinen et al., 2016). In the extended night treatment, phosphorylation states of proteins such as EPSIN1 (EPS1), EPS2, golgin candidate 2 (GC2), ARF GAP domain 7 (AGD7), and AGD8 were found to be altered in SnRK1 mutant or overexpression lines (Fig. 3; Table S2 at Dryad) (Nukarinen et al., 2016). Several of these proteins are important regulators of protein trafficking. For example, EPS1 is involved in protein trafficking at the Golgi network (Song et al., 2006). Collectively, evidence from PPI and phosphoproteome analyses suggests an important role for SnRK1 in regulating cytoskeleton dynamics, chloroplast movement, and protein trafficking. This is not surprising as SnRK1 is a major regulator of cell division, plant growth, and architecture (Baena-González and Hanson, 2017). However, specific studies are needed to identify the direct role of SnRK1 in these processes.

Autophagy machinery

AMPK/Snfl/SnRK1 is a positive regulator of autophagy during nutrient starvation (J. Kim et al., 2011; Soto-Burgos and Bassham, 2017; Coccetti et al., 2018; Herzig and Shaw, 2018). In mammals, the molecular mechanism of AMPK-mediated control of autophagy is understood in great detail (Herzig and Shaw, 2018). During glucose starvation, AMPK activates Unc-51 like autophagy activating kinase (ULK1) through phosphorylating specific serine residues (Egan et al., 2011; J. Kim et al., 2011). Interestingly, in glucose sufficiency, ULK1 is phosphorylated by mTORC1 on another serine residue, which prevents its interaction with AMPK (J. Kim et al., 2011). Thus, the regulation of ULK1 by AMPK and mTORC1 is important in coordinating autophagy according to nutrient availability in mammals. Interestingly, ULK1 is also part of a negative feedback loop of AMPK and autophagy through phosphorylating AMPK subunits (Löffler et al., 2011). Although SnRK1 was found to be a positive regulator of autophagy in Arabidopsis (Chen et al., 2017; Soto-Burgos and Bassham, 2017), molecular understanding of how SnRK1 and autophagy are connected in plants is limited. In Arabidopsis, overexpression of SnRK1α1 enhanced autophagosome formation and phosphorylation of autophagy-related protein 1A (ATG1a) in vivo (Chen et al., 2017). Further, in the Y2H assays, SnRK1α1 showed interaction with ATG1a and ATG13a. However, these interactions could not be confirmed in planta (Chen et al., 2017). Therefore, more studies are needed to identify whether ATG1a is a direct phosphorylation target of SnRK1. In Arabidopsis, SnRK1 phosphorylates autophagy 6 (ATG6) in vitro and promotes autophagy during prolonged carbon starvation (Fig. 2A)}
(Huang et al., 2019). SnRK1 was also found to be a positive regulator of autophagy in response to abiotic and endoplasmic reticulum stress (Soto-Burgos and Basham, 2017). Thus, SnRK1 works as a master regulator of autophagy and nutrient recycling under different environmental conditions.

Proteins involved in chloroplast function and development

SnRK1 is localized in the cytoplasm, nucleus, and chloroplast, and is associated with the endoplasmic reticulum (Fragoso et al., 2009; Bitrián et al., 2011; Tsai and Gazzarrini, 2012; Williams et al., 2014; Chan et al., 2017; Jamsheer K et al., 2018a; Blanco et al., 2019). PPI screening identified the interaction of SnRK1 with enzymes involved in chlorophyll biosynthesis and chloroplast development, such as Genomes uncoupled 4 (GUN4), qberant chloroplast development 4 (ABC4), and RELA/SPOT homolog 3 (RSH3) in Arabidopsis, and protoclorophyllide reductase B (PORB) in rice, suggesting a potential role for SnRK1 in chloroplast development (Figs 2A, 4) (Rohila et al., 2009; Carianopol et al., 2020). Further studies are needed to identify the biological significance of these interactions. In the phosphoproteome analyses, perturbation of SnRK1 signaling altered the phosphorylation states of several proteins involved in chloroplast development and function such as Reduced chloroplast coverage 1 (REC1), REC2, and Curvature thylakoid 1B (CURT1B) (Fig. 3; Table S2 at Dryad) (Cho et al., 2016; Nukarinen et al., 2016). Further studies are needed to delineate how these proteins are connected with the SnRK1 signaling network in plants.

Proteins involved in biotic stress responses

SnRK1 promotes broad-spectrum disease resistance against bacterial and fungal pathogens through promoting JA and salicylic acid (SA) signaling in rice (Filipe et al., 2018). However, the molecular mechanism of SnRK1-mediated resistance against bacterial and fungal pathogens is yet to be determined. Arabidopsis SnRK1 interacts with proteins involved in the biotic stress pathway such as recognition of Peronospora parasitica 13 (RPP13), Phloem protein 2 A5 (PP2A5), and nematode resistance genes HSPRO1 and HSPRO2 (Fig. 2A; Table S1 at Dryad). The tomato SnRK1 interacts with Xanthomonas campestris pv. vesicatoria (Xcv) effector proteins AvrBs1 and AvrBsT (Fig. 5), and was found to be required for AvrBs1–specific induction of the hypersensitive response (Szczesny et al., 2010).

In Arabidopsis, SnRK1 phosphorylates adenosine kinase (ADK) in vitro. ADK is involved in the synthesis of AMP in the salvage pathway, and SnRK1 enhances ADK activity in a non-enzymatic manner. Counterintuitively, reduction in SnRK1 activity enhanced the ADK activity, indicating that SnRK1 and ADK activities are linked in a complex manner (Mohannath et al., 2014). Interestingly, Geminivirus AL2 and L2 proteins inactivate both SnRK1 and ADK to successfully infect plants (Hao et al., 2003; Wang et al., 2003). Synthesis of AMP by ADK might activate SnRK1 during viral infection, and SnRK1 is involved in the regulation of ADK activity (Mohannath et al., 2014). Thus, the SnRK1–ADK module appears to be a part of the innate defense mechanism against viruses in plants. The SnRK1-activating kinases SnAK1 and SnAK2 were initially identified as Geminivirus Rep-interacting kinases (GRIKs) as the expression of GRIKs is enhanced during Geminivirus infection (Kong and Hanley-Bowdoin, 2002). This suggests the activation of SnRK1 signaling during viral attack. SnRK1 in turn phosphorylates AL2 and L2 proteins from many different Geminiviruses (Fig. 5). This phosphorylation was found to reduce the infection of the Cabbage leaf curl virus (CaLCuV) in Arabidopsis (Shen et al., 2014). SnRK1 also phosphorylates Tomato yellow leaf curl China β-satellite (TYLCCNB) βC1, which delays the infection in tomato (Shen et al., 2011). Further, cotton SnRK1 interacts with Cotton leaf curl multian β-satellite (CLCuD) βC1 (Kamal et al., 2019). Thus, AL2, L2,
and βC1 proteins seem to be the conserved substrates of SnRK1 signaling in viruses to negatively regulate infection in the host plants. Another substrate of SnRK1 is *Tomato golden mosaic virus* (TGMV) replication initiator protein (REP). This phosphorylation also negatively regulates TGMV replication and infection (Shen *et al.*, 2018).

Along with viral proteins, SnRK1 also phosphorylates plant proteins involved in viral infection (Figs 2A, 3). In Y2H assays, SnRK1 interacts with remorin 4.1 (REM4.1) and REM4.2 which are involved in promoting susceptibility of plants to Geminiviruses. SnRK1 phosphorylates REM4.1 in *vitro*. This phosphorylation probably down-regulates REM4.1 activity (Son *et al.*, 2014). Interestingly, the phosphorylation states of other remorins involved in antiviral pathways such as REM1, REM1.2, and REM1.3 were found to be altered in SnRK1 mutant lines under extended night treatment (Fig. 3F; Table S2 at Dryad) (Nukarinen *et al.*, 2016). Viruses co-opted the TORC1 pathway to promote the translation of viral proteins (Schepetilnikov and Ryabova, 2018). Thus, negative regulation of TORC1 signaling by SnRK1 is possibly an evolutionarily conserved antiviral mechanism in plants.

**FCS-like zinc fingers and SnRK1 signaling**

Y2H screenings identified promiscuous interaction of Arabidopsis SnRK1 with members of a land plant-specific zinc finger protein family named FCS-like zinc fingers (FLZs) (Arabidopsis Interactome Mapping Consortium, 2011; Nietzsche *et al.*, 2014, 2016; Jamsheer K *et al.*, 2018b; Carianopol *et al.*, 2020). These proteins were earlier known as DUF581 family proteins (Jamsheer K and Laxmi, 2014). The FLZ proteins generally possess conserved intrinsically disordered regions (IDRs) in the N-terminus and a C2-C2 FLZ domain, which cooperates in the association with the SnRK1 complex. Further, the IDRs facilitate specific hetero- and homodimerization of FLZs (Jamsheer K *et al.*, 2018b). Intriguingly, FLZs and SnRK1 share common interacting proteins, which include TFs and metabolic enzymes (Fig. 2B) (Arabidopsis Interactome Mapping Consortium, 2011; Nietzsche *et al.*, 2014, 2016; Jamsheer K *et al.*, 2019). Another crucial common interacting protein is RAPTOR1B, the important regulatory component of the TORC1 (Arabidopsis Interactome Mapping Consortium, 2011). Thus, FLZs are hypothesized to be scaffold proteins that facilitate the recruitment of proteins to the SnRK1 complex (Nietzsche *et al.*, 2014, 2016; Jamsheer K *et al.*, 2018b). Apart from Arabidopsis, the interaction of SnRK1 with several FLZ proteins is reported in rice and wild soybean (Fig. 4). Interestingly, SnRK1 signaling was found to regulate the transcript levels of several FLZ genes in Arabidopsis (Jamsheer K and Laxmi, 2015). Further, specific FLZ proteins (FLZ6 and FLZ10) were found to be involved in negatively regulating the stability of SnRK1α1. This regulation occurs in a negative feedback loop which helps in moderating SnRK1 signaling during sugar starvation and maintaining TORC1 activity in Arabidopsis (Jamsheer K *et al.*, 2018a). More work is needed to identify how FLZ proteins regulate the protein level of SnRK1α1. Nonetheless, the available evidence indicates that FLZs are relevant to the SnRK1 signaling network in plants.

**Proteins involved in other signaling networks**

Interaction of SnRK1 with VIP1, CBL1, and CIPK/SnRK3s along with nutrient transporters and channels indicate their complex connection in controlling Ca2+, stress, and nutrient signaling in plants (Fig. 2A; Table S2 at Dryad). In line with this, phosphorylation states of Ca2+-binding proteins such as calmodulin like 43 (CML43), calnexin 1 (CNX1), annexin 2 (ANN2), and multiple members of the IQ67-domain (IQD) protein family were found to be altered due to perturbation in SnRK1 signaling (Fig. 3; Table S1 at Dryad) (Cho *et al.*, 2016; Nukarinen *et al.*, 2016). Further, SnRK1 is found to interact with ferritin 2 (FER2) and FER3 in the Y2H system (Carianopol *et al.*, 2020). FER2 and FER3 are involved in iron homeostasis and oxidative stress mitigation (Carianopol *et al.*, 2020). SnRK1 also directly interacts with ABA receptors Pyrabactin resistance 1 (PYR1) and PYR1-like 4 (PYL4) and downstream stress proteins in the Y2H system (Fig. 2A) (Carianopol *et al.*, 2020). In line with this, phosphorylation states of stress proteins such as early responsive to dehydration 10 (ERD10) and ERD14 were found to be altered in the snrk1a1/a2 line under extended night treatment (Nukarinen *et al.*, 2016) (Fig. 3). PPI analyses revealed that SnRK1 also interacts with 20S proteasome alpha subunit PAD1, COP9 signalosome subunit 7 (CSN7), ubiquitin-conjugating enzyme 17 (UBC17), UBIQUITIN 3 (UBQ3), and other putative components of the protein ubiquitination system (Figs 2A, 4; Table S1 at Dryad) (Bhalerao *et al.*, 1999; Farrés *et al.*, 2001; Lee *et al.*, 2008; Carianopol *et al.*, 2020). Interestingly, phosphoproteome analyses identified that the phosphorylation states of proteins involved in ubiquitination and the SUMOylation system were altered due to perturbation in SnRK1 signaling, especially during the extended night or submergence stress treatments (Fig. 3; Table S2 at Dryad) (Cho *et al.*, 2016; Nukarinen *et al.*, 2016). These results suggest the possibility that the SnRK1 signaling network might also be involved in the regulation of protein ubiquitination and SUMOylation machinery in plants. Interaction of SnRK1 subunits is also reported with regulators of plant development such as blue-light inhibitor of cryptochromes 2 (BIC2) and tiller angle control 1 (TAC1) in Y2H assays (Fig. 2A) (Carianopol *et al.*, 2020). More targeted studies are needed for elucidation of the biological relevance of these interactions.
Concluding remarks and future perspectives

Studies in different plant systems identified that SnRK1 is a central integrator of diverse environmental signals and coordinating growth. Despite significant evolutionary changes, the central role of SnRK1 as a master regulator of cellular adjustment to sugar starvation is conserved in the plant lineage. Our comprehensive analysis of PPI and phosphoproteomics data revealed an extensive communication of SnRK1 with transcription and translational machinery, protein kinases and phosphatases, protein ubiquitination and SUMOylation machinery, metabolism, cytoskeleton, and protein trafficking. Many of these PPIs were identified in high-throughput screening, especially using Y2H assays. Therefore, more focused studies are needed to verify these interactions in planta. Among the verified PPIs, the biological significance of only limited interactions is known. Thus, a significant portion of the SnRK1 signaling network in plants remains to be studied critically. In our analysis, the communication of SnRK1 with many signaling and metabolic pathways seems to be reciprocal. For example, sugar status is an important regulator of SnRK1 activity. At the same time, SnRK1 appears to be a regulator of chloroplast development, photosynthesis, and starch metabolism. T6P is a potent regulator of SnRK1 signaling in plants. At the same time, SnRK1 signaling appears to regulate T6P levels. Thus, through different regulatory communications, SnRK1 works as a central hub complex involved in the regulation of growth and resilience of plants in different environmental conditions.

Our analysis using PPI and phosphoproteomic data reveals many potential novel components of SnRK1 signaling in plants. In-depth functional analysis of these components in the context of SnRK1 signaling will broaden our understanding of the molecular basis of the nutrient-dependent control of plant growth and developmental plasticity in plants.

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Conflict of interest
The authors report no conflict of interest.

Data availability
A compiled list of SnRK1 interaction and phosphorylation data, and SnRK1 phosphoproteome data are openly available at the Dryad Digital Repository https://doi.org/10.5061/dryad.c2tqz6178.

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