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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.*,

2016). Thus, AMPK works as a sensor of several types of nutrients in eukaryotes.

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).

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

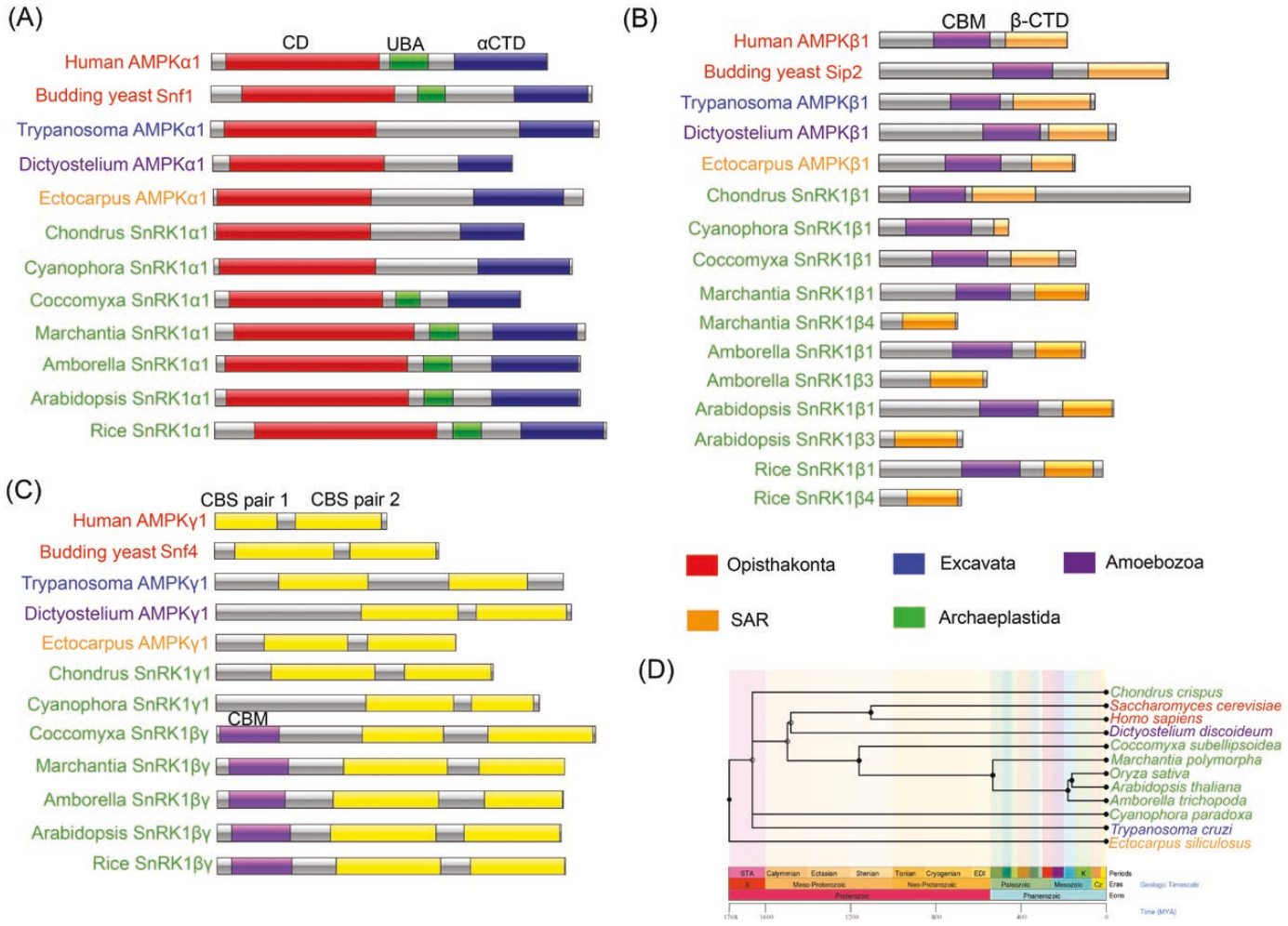

Fig. 1. Representative domain composition of SnRK1/AMPK/Snf1 subunits from different plant species in comparison with other eukaryotic supergroups. (A–C) The domain composition of α kinase and β, γ, βγ regulatory subunits. The different eukaryotic supergroups are distinguished by colors. The protein sequences were retrieved through BLAST searches using Arabidopsis SnRK1 subunits as the query. IBS webserver was used to annotate domains utlizing 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 SnRK1α1 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 SnRK1α1 *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; Nukarinen *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 (Jamsheer 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 relocalization 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, SnRK1α1 is translocated to the nucleus which is important for regulating gene expression. Myristoylation of SnRK1β2 was found to negatively regulate the nuclear translocation of SnRK1α1 (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 (Ávila-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 (Ávila-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 adenylate 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 adenylate 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 sugar (sucrose 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.*, 2018*a*). 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 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.*, 2018*a*). 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), *Dictyostelium purpureum* (Amoebozoa), and *Ectocarpus 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; Nukarinen *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

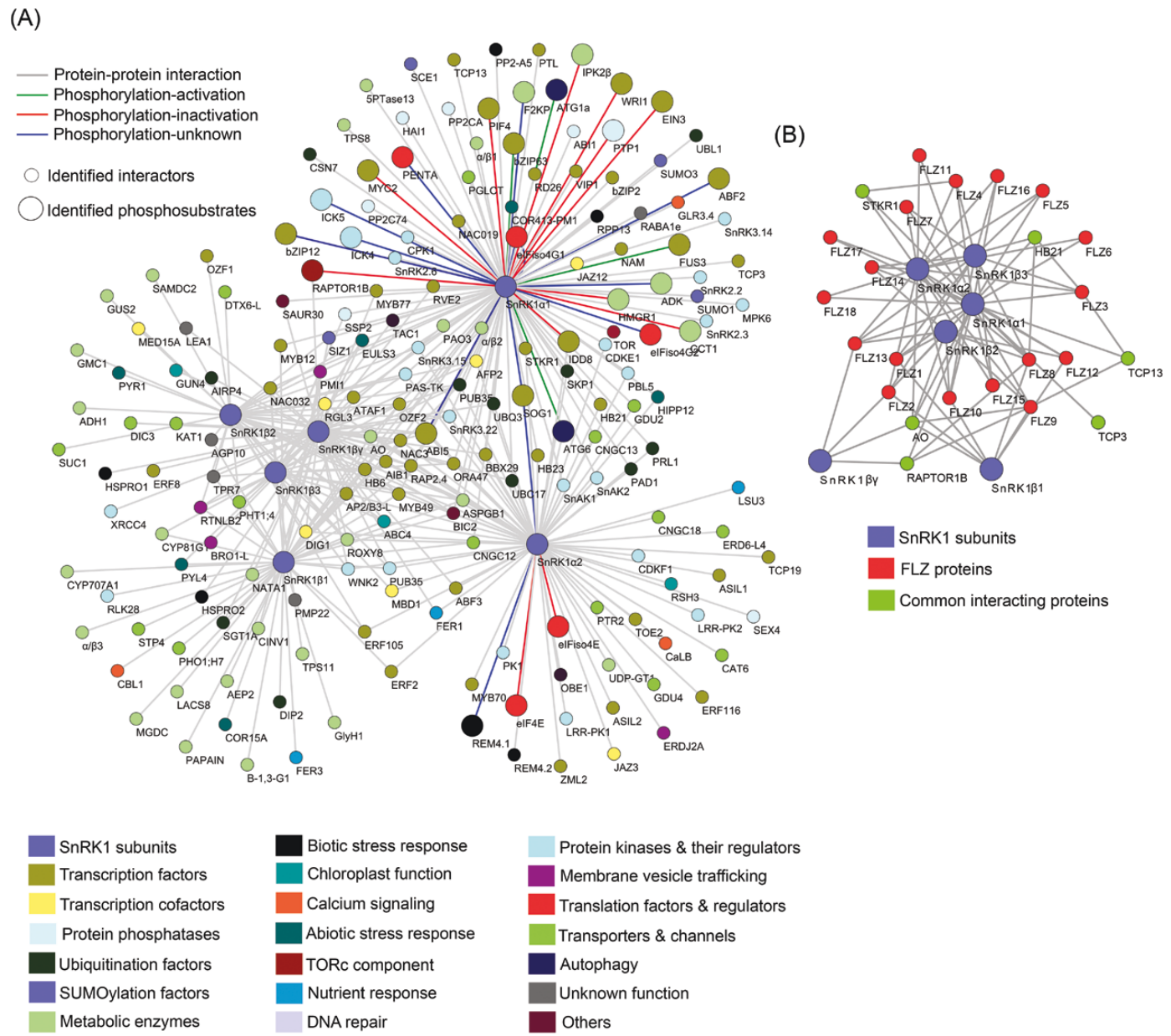

Fig. 2. The Arabidopsis SnRK1 signaling network based on direct protein–protein interaction and phosphorylation. (A) Interaction and phosphorylation network of Arabidopsis SnRK1 subunits. (B) Interaction subnetwork of SnRK1 subunits and FLZ proteins, highlighting the common interacting proteins. The 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 AIV 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 S1available at Dryad for more details.

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 (*snrk1α1*^{K48M}) 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 *snrk1α1* 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 *snrk1α1* knockout mutant. This *snrk1α1/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 unchartered 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. *etfqo* 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

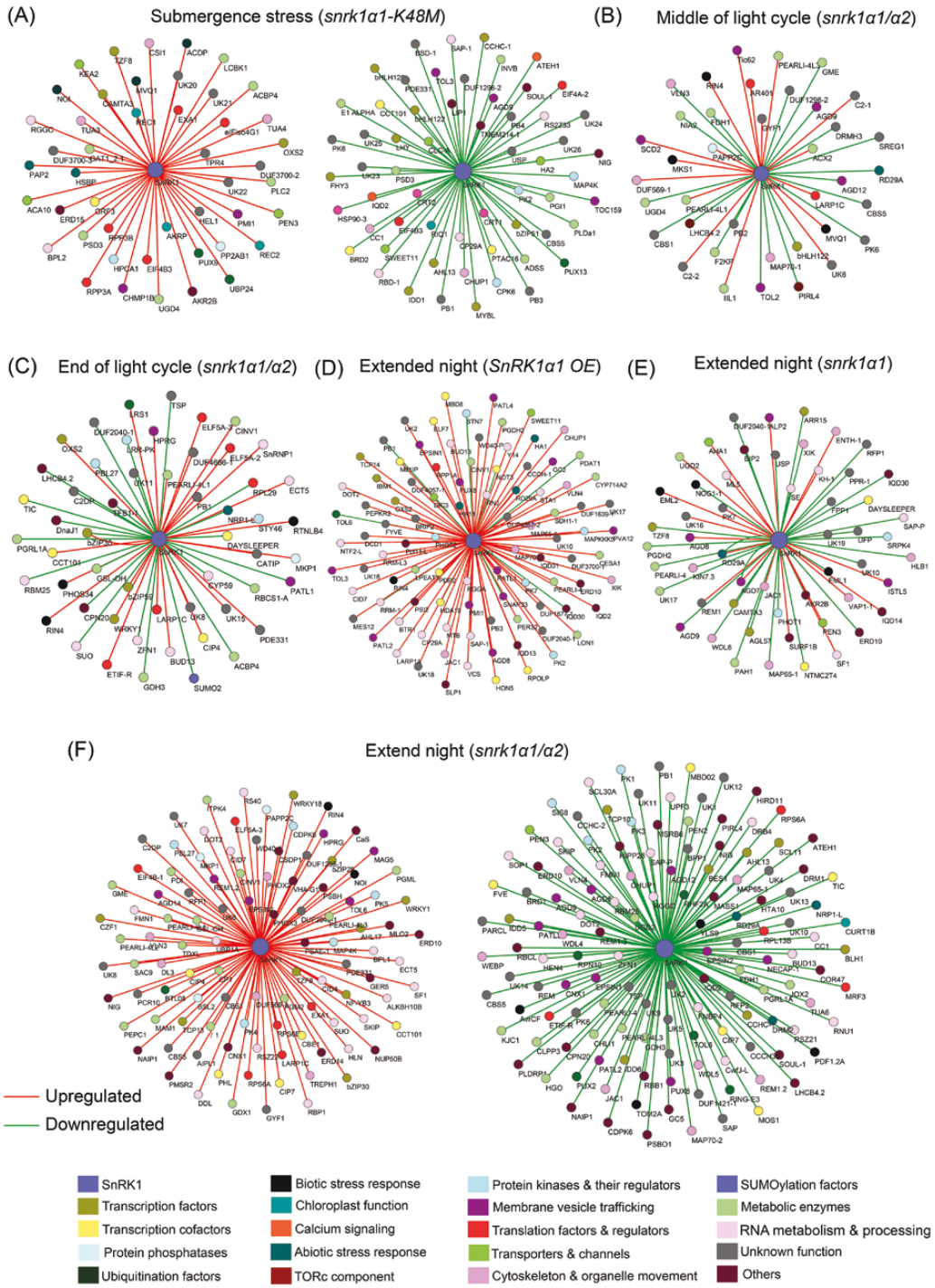

Fig. 3. Compiled network of proteins with altered phosphorylation states due to the perturbation in SnRK1 signaling in Arabidopsis. The phosphoproteomic data of Arabidopsis SnRK1 were retrieved from two studies (Cho *et al.*, 2016; Nukarinen *et al.*, 2016). A dominant-negative mutant of SnRK1α1 (*snrk1α1*^{K48M}) was to used 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 the 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 *snrk1α1*^{K48M} during submergence stress. (B and C) The network of proteins with altered phosphorylation state 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.

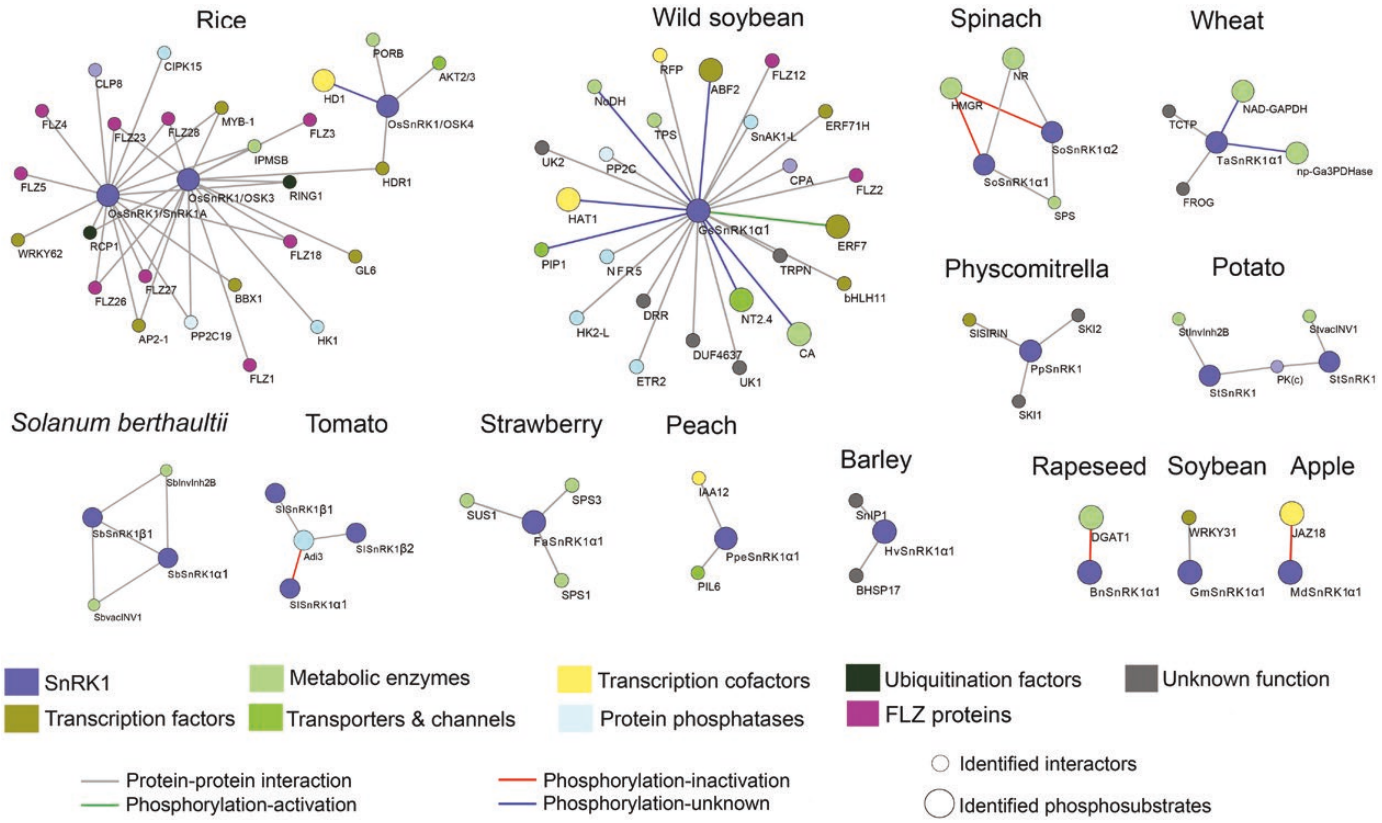

Fig. 4. The SnRK1 signaling network in different plants based on direct protein–protein interaction and phosphorylation. The interaction and phosphorylation data of SnRK1 subunits in different plants were retrieved from protein–protein interaction databases (BioGRID v3.5.185, STRING v11.0, and IntAct v4.2.14) and literature mining. The interactors were annotated using Phytozome v12.1 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.

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 plants (Fig. 2A). In line with this, SnRK1 and ABA signaling were found to regulate a common set of stress-responsive genes in a synergistic manner (Rodrigues *et al.*, 2013).

PPI analyses revealed that SnRK1 subunits interact with multiple members of Apetala 2 (AP2), Teosinte branched1/Cincinnata/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 WRI1 (Table 1). Through this regulation, lipid biosynthesis is negatively regulated in

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

Interactor	Interactor category	Outcome of phosphorylation	Pathway	References
bZIP63 (Arabidopsis)	Transcription factor	Enhanced the heterodimerization with S1-bZIPs *in planta*	Transcription of sugar starvation-responsive genes	Mair *et al.* (2015)
WRI1 (Arabidopsis)	Transcription factor	Enhanced the degradation of WRI1 in cell-free degradation assay	Seed fatty acid biosynthesis	Zhai et al. (2017*a*, 2018)
PIF4 (Arabidopsis)	Transcription factor	Possibly promotes degradation as co-expression of SnRK1α1 reduced the PIF4 stability *in planta*	Thermomorphogenesis	Hwang *et al.* (2019)
FUS3 (Arabidopsis)	Transcription factor	Possibly promotes stability as incubation of protein extract from *35S:SnRK1α1-HA* seedlings enhanced the stability of FUS3	Developmental phase transitions Seed development	Tsai and Gazzarrini (2012); Chan *et al.* (2017)
eIF4E eIFiso4E (Arabidopsis)	Protein synthesis machinery	Possibly down-regulates the activity as co-expression of SnRK1α2-CD inhibited the ability of eIF4E/eIFiso4E to complement *eif4e* budding yeast mutant in growth assay and polysome formation	Translation initiation	Bruns *et al.* (2019)
PTP1 (Arabidopsis)	Protein phosphatase	Reduced the interaction with MPK6 *in planta*	MPK6 signaling during submergence	Cho *et al.* (2016)
KRP6 KRP7 (Arabidopsis)	CDK inhibitor	Possibly down-regulates the activity as phosphomimetic KRP6 ($KRP6^{T152D}$) showed reduced interaction with CycD3;1 in Y2H assay	Cell cycle	Guérinier *et al.* (2013)
SPS (Spinach)	Carbohydrate metabolism	Reduced the *in vitro* enzyme activity	Sucrose biosynthesis	Sugden *et al.* (1999)
NR (Spinach)	Nitrogen metabolism	Reduced the *in vitro* enzyme activity	Nitrate assimilation	Sugden *et al.* (1999)
DGAT1 (Brassica)	Lipid metabolism	Reduced the *in vitro* enzyme activity	Triacylglycerol biosynthesis	(Caldo *et al.*, 2018)
HMGR (Spinach, Arabidopsis)	Lipid metabolism	Reduced the *in vitro* enzyme activity	Sterol and isoprenoid biosynthesis	Sugden *et al.* (1999); Robertlee *et al.* (2017)

sugar-limited conditions in plants (Zhai *et al.*, 2017*a*). 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.*, 2017*b*, 2018). T6P weakened the interaction of SnRK1α1 with the activating kinase SnAK2 *in vitro*. In the SnRK1 activity assay using seedling extracts, T6P-mediated inhibition of SnRK1 activity was found to be abolished in the double mutant of SnAKs (Zhai *et al.*, 2018). Thus, the available evidence suggests an important role for the T6P–SnRK1–WRI1 signaling axis as a homeostatic control mechanism to balance lipid biosynthesis according to sugar availability in plants. However, more genetic studies using double and triple mutants will be required to verify the relevance of this signaling axis *in planta*. Using Y2H assay, the interaction of other AP2 TFs such as target of early activation tagged (Eat) 2 (TOE2), ethylene response factor 2 (ERF2), ERF8, ERF105, ERF116, octadecanoid-responsive AP2/ERF-domain transcription factor 47 (ORA47), and related to AP2.4 (RAP2.4) with SnRK1 is identified in Arabidopsis (Fig. 2A) (Arabidopsis Interactome Mapping Consortium, 2011; Carianopol *et al.*, 2020). Interaction of SnRK1 with AP2 TFs of rice and wild soybean was identified in Y2H screens (Fig. 4) (Ding *et al.*, 2009; Song *et al.*, 2019). AP2 is a large and ubiquitous TF family in higher plants with important roles in development, and hormone and stress signaling (Licausi *et al.*, 2013). The widespread interactions of AP2 TFs with SnRK1 suggest the presence of an SnRK1–AP2 signaling network in plants. 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 Suppressor 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

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 SnAK2 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 *snrk1α1/α2* or *snrk1α1*^{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 SnRK1α 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 ABI5-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), PWWP 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 Opisthakonta, the role of AMPK/Snf1 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/Snf1, 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), STARIK 1 (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 *SnRK1α1* 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 SnRK1α1 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 Tuberous 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 SnRK1α1 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 (Bruns *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 (KRP6) and KRP7 *in vitro* (Fig. 2A; Table 1). In KRP6, 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, KRPs 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 SnRK1α 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 (Thelander *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 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 (Piattoni *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 (*snrk1α1*^{K48M}) 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 SnRK1.1^{K48A} (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, which suppresses the TOR activity, presumably through the direct phosphorylation-mediated inhibition of RAPTOR (Davie *et al.*, 2015). In nitrogen sufficiency, TORC1 suppresses Snf1 activity by down-regulating Thr210 phosphorylation in the activation loop of the kinase subunit (Orlova *et al.*, 2006). However, how TORC1 suppresses Snf1 activity is yet to be determined. Recently, TORC1 was found to directly inhibit AMPK signaling through the phosphorylation of an evolutionarily conserved serine residue in the AMPK kinase subunit in mammals and fission yeast (Ling *et al.*, 2020). Thus, reciprocal interaction between TORC1 and Snf1/AMPK/SnRK1 may be critical for growth adjustments according to nitrogen availability in eukaryotes. In Arabidopsis, nitrogen starvation moderately increased SnRK1 activity *in vitro* (Nunes *et al.*, 2013). However, the physiological significance of SnRK1 signaling in nitrogen starvation is yet to be identified in plants.

Overexpression of Arabidopsis *SnRK1α1* in mesophyll protoplasts induced the expression of genes involved in amino acid catabolism and suppressed genes involved in amino acid biosynthesis. Specifically, the expression of genes involved in the degradation of asparagine, tyrosine, leucine, etc. and 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.*, 2018*a*). 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).

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 WRI1 (Zhai *et al.*, 2017*b*). 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.*, 2017*a*, 2018). The overexpression and RNAi-mediated suppression of the *SnRK1α1* level led to reduced triacylglycerol (TAG) levels in the seeds of Arabidopsis (Zhai *et al.*, 2017*a*). At the molecular level, SnRK1-mediated phosphorylation promotes the degradation of WRI1, the positive regulator of fatty acid synthesis in seeds (Zhai *et al.*, 2017*a*, 2018). Rapeseed SnRK1 phosphorylates and inactivates diacylglycerol acyltransferase 1 (DGAT1) *in vitro* (Fig. 4; Table 1). DGAT1 catalyzes the final

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 (HMGR) in spinach and Arabidopsis *in vitro*. HMGR 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 phosphorylcholine 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 is reported 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 SnRK1α1 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 Föller, 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; H7) 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; Nukarinen *et al.*, 2016). More targeted studies are needed to identify the direct/indirect 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,

2000). Studies in mammalian systems revealed an important role for AMPK in controlling cytoskeleton organization. In osmotic stress conditions, AMPK is involved in the reorganization of the actin cytoskeleton in epithelial cells (Miranda *et al.*, 2010). In breast cancer cells, alteration of AMPK activity using pharmacological approaches affected the activity of the actin-severing protein, cofilin, and microtubule stability (Chakrabarti *et al.*, 2015). In line with this, interactome analysis revealed that AMPK subunits interact with a large number of proteins involved in the regulation and organization of actin cytoskeleton in pancreatic β-cells (Moon *et al.*, 2014). Further, in the screening of direct phosphorylation targets of AMPK, human AMPKα2 was found to phosphorylate proteins involved in cytoskeletal reorganization (Banko *et al.*, 2011). Although a direct role for SnRK1 in regulating the cytoskeleton machinery is yet to be established, phosphoproteome analyses revealed that perturbation in SnRK1 signaling affects the phosphorylation states of core cytoskeleton proteins and their regulators (Cho *et al.*, 2016; Nukarinen *et al.*, 2016) (Fig. 3; Table S2 at Dryad). Notably, the phosphorylation of tubulin alpha (TUA), villin (VLN), microtubule-associated protein 70 (MAP70) family proteins, etc. was found to be altered due to perturbation of SnRK1 activity during submergence and extended night treatment (Fig. 3; Table S2 at Dryad) (Cho *et al.*, 2016; Nukarinen *et al.*, 2016). Intriguingly, SnRK1 signaling is also connected with proteins involved in the regulation of chloroplast photorelocation movement. In Y2H assays, SnRK1 interacts with Plastid movement impaired 1 (PMI1), a plant-specific protein involved in the blue light-mediated regulation of chloroplast movement (Fig. 2A) (DeBlasio *et al.*, 2005; Carianopol *et al.*, 2020). Further, the phosphorylation states of Chloroplast unusual positioning 1 (CHUP1), a chloroplast outer membrane actin-binding protein essential for chloroplast photorelocation movement, and THRUMIN1, an actin-bundling protein involved in the regulation of chloroplast movement, were found to be altered due to perturbation in SnRK1 signaling (Oikawa *et al.*, 2003; Whippo *et al.*, 2011; Cho *et al.*, 2016; Nukarinen *et al.*, 2016). Similarly, phosphorylation states of several other key proteins involved in the regulation of cytoskeleton and chloroplast movement were found to be altered in response to perturbation in SnRK1 signaling (Fig. 3; Table S2 at Dryad) (Cho *et al.*, 2016; Nukarinen *et al.*, 2016).

The role of AMPK and Snf1 in regulating the trafficking of glucose transporters especially under glucose starvation is well known (O'Donnell and Schmidt, 2019). Although the direct molecular connection is yet to be established, the PPI screens and phosphoproteome analyses revealed a potential role forSnRK1 in regulating protein trafficking (Figs 2A, 3; Table S1, S2 at Dryad) (Chen *et al.*, 2012; Cho *et al.*, 2016; Nukarinen *et al.*, 2016; Carianopol *et al.*, 2020). Arabidopsis SnRK1 interacts with reticulan-like protein B2 (RTNLB2) which is involved in the transport of immune receptor flagellin-sensitive 2 (FLS2) to the plasma membrane and endoplasmic reticulum 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/Snf1/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 Bassham, 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.*, 2018*a*; 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), qberrant chloroplast development 4 (ABC4), and RELA/SPOT homolog 3 (RSH3) in Arabidopsis, and protochlorophyllide 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 multan* β-*satellite* (CLCuD) βC1 (Kamal *et al.*, 2019). Thus, AL2, L2,

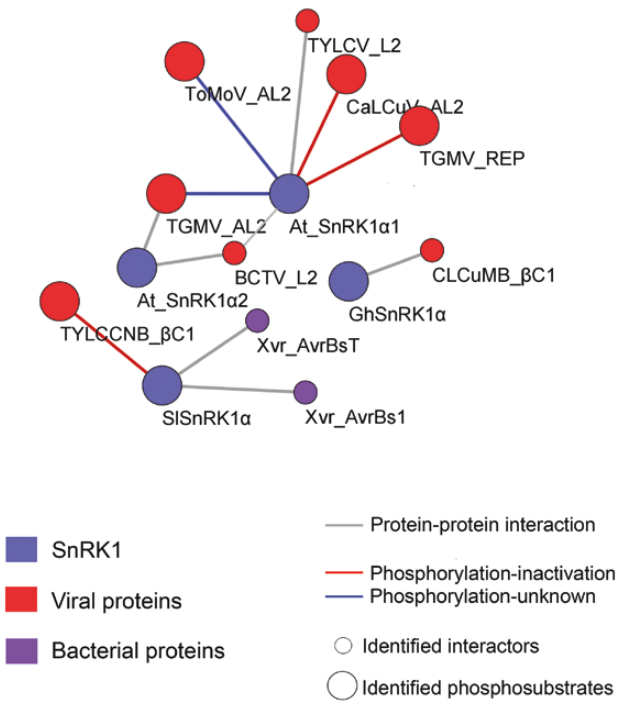

Fig. 5. The interaction and phosphorylation network of SnRK1 with viral and bacterial proteins. The data of viral and bacterial proteins showing interaction or phosphorylation by SnRK1 in different plants were retrieved through literature mining. The network was visualized by Cytoscape v3.8.0. Color keys were used to differentiate SnRK1, viral, and bacterial proteins, and to differentiate interaction and phosphorylation. The abbreviations in the figure are as follows: At, *Arabidopsis thaliana*; Sl, *Solanum lycopersicum*; Gh, *Gossypium hirsutum*; Xcv, *Xanthomonas campestris* pv. *vesicatoria*; TYLCCNB-β, *Tomato yellow leaf curl China virus* β-satellite; BCTV, *Beet curly top virus*; TGMV, *Tomato golden mosaic virus*; ToMoV, *Tomato mottle virus*; TYLCV, *Tomato yellow leaf curl virus*; CalCuv, *Cabbage leaf curl virus*; CLCuMB-β, Cotton leaf curl Multan β-satellite. Please refer to Table S1 available at Dryad for more details.

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.*, 2018*b*; 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-terminyus 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.*, 2018*b*). 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.*, 2018*b*). 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.*, 2018*a*). 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 Ca^{2+}, stress, and nutrient signaling in plants (Fig. 2A; Table S2 at Dryad). In line with this, phosphorylation states of Ca^{2+}-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 *snrk1α1/α2* 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; Farrás *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 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 of SnRK1 phosphoproteome data are openly available at the Dryad Digital Repository https://doi.org/10.5061/dryad.c2fqz6178.

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