Current Opinion in Plant Biology - Issue on Cell signaling and gene regulation 2019.

SnRK1 activation, signaling, and networking for energy homeostasis

Nathalie Crepin[a] and Filip Rolland[a,b]

[a]Laboratory for Molecular Plant Biology, Biology Department, KU Leuven, Kasteelpark Arenberg 31, 3001 Heverlee-Leuven, Belgium (nathalie.crepin@kuleuven.be, filip.rolland@kuleuven.be).

[b]Corresponding author

Abstract

The SnRK1 kinases are key regulators of the plant energy balance, but how their activity is regulated by metabolic status is still unclear. While the heterotrimeric kinase complex is well conserved between plants, fungi and animals, plants appear to have modified its regulation to better fit their unique physiology and lifestyle. The SnRK1 kinases control metabolism, growth and development, and stress tolerance by direct phosphorylation of metabolic enzymes and regulatory proteins and by extensive transcriptional regulation. Diverse types of transcription factors have already been implicated, with a well-studied role for the heterodimerizing group C and group S1 bZIPs. SnRK1 is also part of a more elaborate metabolic and stress signaling network, which includes the TOR kinase and the ABA-signaling SnRK2 kinases.

Introduction

Maintenance of cellular and organismal energy homeostasis is obviously vital to all living beings, but particularly challenging for autotrophic and sessile organisms. Plants are typically exposed to combinations of biotic and abiotic stress conditions, which directly or indirectly affect photosynthesis, respiration, or carbon allocation. Upon activation by such energy-depleting stress conditions, SnRK1 (SNF1-related kinase1), similar to its opisthokont orthologs Sucrose Non-fermenting1 (SNF1) in yeast and AMP-activated kinase (AMPK) in animals, downregulates energy-consuming anabolic processes, while inducing catabolic reactions, thereby ensuring that resources are optimally used and redirected in support of stress tolerance and survival [1,2]. Plants also need to deal with diurnal fluctuations, coordinate source and sink activities, and undergo different developmental transitions that are associated with important changes in metabolic activities and requirements. Consistently, SnRK1 also controls many different aspects of plant development, from embryogenesis and germination to flowering and senescence [3,4]. Still, many questions remain about how SnRK1 controls and

coordinates such diverse processes, and how exactly its activity is regulated in response to metabolic status. Here, we highlight some of the latest insight in both the upstream regulatory and downstream signaling mechanisms and discuss how SnRK1 functions in a more intricate network with Target of Rapamycin (TOR) and abscisic acid (ABA) signaling.

SnRK1 regulation: (a) pretty complex

The eukaryotic AMPK/SNF1/SnRK1 protein kinases operate as heterotrimeric complexes with a catalytic α subunit and regulatory β and γ subunits (Figure 1) [1,5-8]. The catalytic α subunits (SnRK1α1/KIN10 and SnRK1α2/KIN11 in Arabidopsis) consist of an N-terminal Ser/Thr kinase domain linked to a C-terminal regulatory domain, which interacts with the regulatory subunits. Phosphorylation of a conserved threonine (T) residue in the catalytic domain 'T-loop' is a prerequisite for kinase activity. The β subunits (SnRK1β) typically contain a variable N-terminal domain, which is myristoylated, a central carbohydrate-binding module (CBM), and a C-terminal domain for binding of the α and γ subunit. The β subunits thereby act as the complex scaffold, but in addition control kinase activity, localization, and substrate specificity. The γ subunits, finally, typically comprise four highly conserved cystathionine-β synthase (CBS) motifs that can bind adenine nucleotides and hence function as the cellular energy-sensing module of the complex. Green plants, however, use a unique hybrid βγ subunit (SnRK1βγ) with an additional N-terminal CBM as their canonical γ subunit (Figure 1), and also encode a plant-specific β subunit isoform (SnRK1β3 in *Arabidopsis*), which lacks the N-terminal region and CBM but still assembles into SnRK1 complexes [9**,10].

Studies on animal AMPK and yeast SNF1 have been very useful for SnRK1 research, but despite sharing the overall structure and protective function against energy deficit with its opisthokont counterparts, some striking differences also point to regulatory divergence and plant-specific SnRK1 regulatory mechanisms. For example, AMPK and SNF1 are regulated by nucleotide charge, with AMP and/or ADP competing with ATP for binding to the regulatory γ subunit. In AMPK, this results in a conformational change with dissociation of an auto-inhibitory domain (AID) from the catalytic domain and subsequent allosteric activation, inhibition of T-loop dephosphorylation, and promotion of T-loop phosphorylation [6,7]. SnRK1, however, does not appear to be directly activated by reduced nucleotide charge, consistent with mutations of key residues in the βγ subunit nucleotide binding sites and the interacting α subunit linker and lack of an AID [9**-11]. Instead of the AID, SnRK1α harbors a ubiquitin-associated (UBA) domain (Figure 1), which was recently reported to promote T-loop phosphorylation by upstream kinases and maintain catalytic activity [12].

While reversible T-loop phosphorylation is key in AMPK, with phosphorylation levels typically correlating well with (and typically used as a readout of) kinase activity, this correlation is far less clear

in plants [1,13*,14]. The upstream SnRK1-activating kinases SnAK1 and SnAK2 (originally identified as geminivirus Rep protein-interacting kinases or GRIKs) are essential, but appear to auto-phosphorylate/activate, although they are also cross-phosphorylated and inactivated by SnRK1 in an apparent negative feedback loop (Figure 2) [15-17]. SnRK1 also shows significant auto-phosphorylation [13*], suggesting that the upstream kinases may be required for initial phosphorylation and activation of newly synthesized SnRK1 proteins, at least in the proliferating (young and infected) tissues where they are expressed [15]. De-phosphorylation therefore may be a more important regulatory mechanism [14], although recombinant SnRK1 was also reported to be insensitive to dephosphorylation *in vitro* [9**]. Additional upstream kinases (and phosphatases) will likely be identified in different tissues and conditions.

Over-expression of the SnRK1α subunit is sufficient to confer high and specific SnRK1 activity in leaf cells and transgenic plants [13*]. Such complex-independent activity and significant auto-phosphorylation suggest a default activation of the plant SnRK1 catalytic subunit [1]. This also implies that, rather than being activated upon energy deficit like AMPK and SNF1, SnRK1 is repressed under conditions of energy abundance. This makes perfect sense and is likely more reliable for sessile and autotrophic organisms, that need to cope with large and unexpected fluctuations in carbon and energy supplies. Interestingly, plants seem to more generally prefer negative regulation, as illustrated by the many hormone signalling pathways that make use of (often ubiquitination and proteasome-mediated) inactivation and removal of repressor proteins. This apparently enables faster downstream responses [18], but more extensive (mathematical) modeling and experimentation may confirm additional benefits of such systems for plants.

In line with this hypothesis, sugar-phosphates, such as glucose-6-phosphate, glucose-1-phosphate, and trehalose-6-phosphate (T6P), were reported to inhibit SnRK1 activity (Figure 2) [19,20]. While T6P, an intermediate of trehalose metabolism absent in vertebrates, has dramatic effects on plant growth and development, its exact function has long remained elusive, not least because of its low physiological concentrations [21]. T6P acts as a proxy for sucrose levels [21] and already inhibits SnRK1 at low μM concentrations, apparently requiring an unknown proteinaceous factor that is only present in actively growing tissues [19]. However, T6P was recently reported to directly bind to the catalytic SnRK1α subunits (*in vitro*) and to interfere with upstream SnAK/GRIK kinase interaction and T-loop phosphorylation [22*]. It is not yet clear whether the upstream kinases are the elusive missing factor. It will be important to corroborate these findings using complementary biochemical (binding) studies and genetic approaches and to identify the exact T6P binding site(s) and mode(s) of action, as (spatiotemporally) targeted alteration of T6P levels and chemical intervention with T6P analogs have also demonstrated their potential to improve both crop yield and stress resistance [23,24].

In mammals, glycogen binding to the β subunit CBM was also reported to allosterically inactivate AMPK, but in plants it remains unclear whether the SnRK1 CBMs can bind starch (the plant analogue

of glycogen) or other carbohydrates [9**,25]. Maltose (a starch breakdown product) was recently also reported to bind to and activate the SnRK1 complex, involving the CBM of the plant hybrid βγ subunit [26]. The βγ subunit, which also acquired a critical role in pollen hydration and germination [27], recruited its CBM around the appearance of the chloroplastidal green plants and hence the rewiring of cytosolic storage to chloroplastic starch metabolism [10]. Chloroplast (or amyloplast) localization of the SnRK1 complex was also reported [25,26], although this is yet to be confirmed by other labs. Phosphoproteomics also identified SnRK1-dependent chloroplast protein phosphorylation, but this regulation may be indirect [28*]. Different studies point to the existence of both cytosolic and nuclear SnRK1 complexes and (most likely β subunit-mediated) association with membranes, but also more punctate localisations have been observed [1]. A detailed microscopic analysis recently revealed the dynamic localisation of the SnRK1α subunit at the endoplasmic reticulum (ER) membrane and in the nucleus [29]. This is consistent with our findings that the α subunit translocates to the nucleus in response to energy stress to activate gene expression, and that the β subunits negatively regulate this response by myristoylation-mediated cytoplasmic retention (unpublished). Interestingly, the cereal SnRK1-interacting negative regulator proteins (SKINs) similarly antagonize the function of the seed-specific SnRK1A by cytoplasmic retention in response to ABA [30], confirming that localization is another important regulatory mechanism. Remarkably, a number of recent studies suggest that AMPK is also able to sense glucose availability independently of nucleotide charge and that this involves the glycolytic enzyme aldolase (as a metabolic sensor of its substrate fructose-1,6-bisP) and an upstream kinase-containing AMPK-activating complex at the surface of the lysosome [31]. In plants, tonoplast association has not been reported.

SnRK1 also restrict its own activity by sumoylation- and ubiquitination-mediated degradation as an efficient feedback mechanism to avoid a persistent stress response [32]. A similar feedback mechanism involves two ABA- and SnRK1-induced members of the DUF581 (domain of unknown function 581) containing small FLZ (FCS-like zinc finger) proteins, previously identified as possible (downstream) SnRK1 adaptor proteins [33,34]. They interact with SnRK1 at the ER and appear to repress SnRK1 signaling in part by regulating SnRK1α protein levels [35]. Finally, similarly to AMPK and SNF1, SnRK1 activity is possibly further fine-tuned by additional post-translational modifications (including non-T-loop phosphorylation and acetylation) and was reported to be redox regulated *in vitro* [1,36].

SnRK1 activity: protein phosphorylation and transcriptional reprogramming

Upon metabolic stress, SnRK1 elicits an energy-saving program to maintain or restore homeostasis using two major modes of action: (i) direct phosphorylation and modification of key metabolic enzymes and regulatory (e.g. signaling) proteins and (ii) extensive transcriptional reprogramming [1]. Enzymes involved in primary metabolism (such as sucrose-phosphate synthase, nitrate reductase, and HMG-CoA reductase) were amongst the earliest studied SnRK1 targets and are typically found to be inactivated

upon phosphorylation [1,37]. Quantitative phosphoproteomics analyses are now identifying more putative *in vivo* targets at a larger scale, for example consistent with SnRK1-mediated redirection of carbon flux towards glycolysis and respiration or fermentation under light and oxygen deprivation conditions [28*,38,39]. Importantly, direct SnRK1 phosphorylation events and their physiological significance need to be confirmed by follow-up studies.

In addition to targeting enzymes and regulatory proteins post-translationally, SnRK1 signaling also triggers a transcriptional switch, inducing the expression of genes involved in the major catabolic pathways and autophagy, while repressing genes associated with various energy-consuming processes, including ribosome biosynthesis [13*]. Over 1000 SnRK1-regulated (induced and repressed) genes were identified using transient overexpression of *Arabidopsis* SnRK1α1/KIN10 in leaf mesophyll cells as a first step towards analysis of its impact on gene expression, also confirming a role for SnRK1 beyond mere metabolic regulation [13*]. However, the full complement of primary (protein synthesis independent) SnRK1 target genes in intact plants and different (including sink and meristematic) tissues and conditions still remains to be uncovered.

Part of this transcriptional reprogramming is mediated by basic region/leucine zipper motif (bZIP)-type transcription factors (TFs), with a key role for the group C and group S1 bZIPs as signaling hubs coordinating plant development and stress responses [40]. SnRK1 hyperphosphorylation of the group C bZIP63 strongly enhances its ability to homodimerize or heterodimerize with group S1 bZIPs and bind to DNA [41*]. This then also recruits SnRK1 to target promoters where the C/S1-bZIP-SnRK1 complex interacts with the histone acetylation machinery to remodel chromatin and facilitate transcription. This, for example, induces branched-chain amino acid catabolism as an alternative pathway to feed into mitochondrial respiration and generate ATP from non-carbohydrate sources in the absence of photosynthesis [42*]. The group S bZIP11 was also found to directly activate the expression of *INDOLE ACETIC ACID3/SHORT HYPOCOTYL2* (*IAA3/SHY2)*, a key negative regulator of auxin signaling and PIN-FORMED (PIN; auxin transport facilitator) expression, providing a straightforward mechanism to control (root) growth in response to energy supply [43*]. It is not yet clear, however, whether this is mediated by a SnRK1-phosphorylated (for example group C bZIP TF) partner. Finally, and in line with an intuitive and previously recognized role for SnRK1 in metabolic entrainment of the plant clock [44-46], bZIP63 was also found to act downstream of T6P and SnRK1, directly binding to the afternoon-phased PSEUDO RESPONSE REGULATOR7 (PRR7) promoter [47*]. Consistent with their key function in SnRK1 signaling and metabolic homeostasis, C/S1 bZIP TF expression is also fine-regulated by SnRK1 at the level of transcription and by sucrose-induced repression of translation (SIRT), involving small upstream open reading frames [42*,48].

A steadily increasing number of other types of TFs, involved in diverse metabolic and developmental processes, are now also found to be regulated by SnRK1. Phosphorylation of the INDETERMINATE DOMAIN (IDD)-containing TF IDD8, for example, results in decreased transcriptional IDD8 activity and inhibition of flowering [49]. SnRK1 was also reported to phosphorylate FUSCA3 (FUS3) *in vitro*

and enhance its protein stability (in a cell-free system). Genetic analyses suggest that FUS3 partially mediates SnRK1's repressive effect on the embryonic-to-vegetative (germination) and vegetative-to-reproductive (flowering) phase transitions, but that FUS3 phosphorylation and SnRK1 positively regulate embryogenesis [50,51]. Conversely, SnRK1 phosphorylation promotes the proteasomal degradation of WRINKLED1 (WRI1), reducing lipid biosynthesis [52]. Similarly, SnRK1 delays senescence by phosphorylation and concomitant destabilization of the ETHYLENE INSENSITIVE3 (EIN3) TF [53]. In addition, a number of TFs interact with SnRK1 without evidence for phosphorylation yet, as most recently reported for the STOREKEEPER RELATED1/G-element Binding Protein (STKR1) [1,54]. The previously mentioned SnRK1-interacting DUF581 proteins were also proposed to mediate downstream SnRK1 signaling by acting as tissue- and (stress) stimulus-specific scaffolds for diverse targets, including a number of TFs and DELLA proteins, also reallocating the kinase to specific regions in the nucleus [33,34,55]. Interestingly, part of the gene expression regulation by SnRK1 may be mediated by miRNAs [56].

SnRK1 is part of an intricate metabolic and stress signaling network

SnRK1 acts in an intricate network, that also includes both the generally growth-stimulating and antagonistic TOR pathway and abscisic acid (ABA)-mediated stress signaling (Figure 2). Intense research in the past ten years or so has confirmed a central role for the TOR kinase in the regulation of plant metabolism, development, and stress physiology [57-59]. It has highly conserved functions in the direct control of protein synthesis, autophagy and growth processes in all eukaryotic organisms, and in plants appears to integrate macronutrient, energy (glucose), light, and hormone (notably auxin) signaling [57-59]. SnRK1 is found to function upstream of TOR and appears to confer part of its effects through inhibition of TOR signaling. Profiling of the extensive glucose-induced TOR-mediated transcriptional reprogramming revealed some overlap with (oppositely regulated) SnRK1 target genes [60]. Later studies confirmed inhibition of TOR signaling by SnRK1 under energy deprivation conditions, involving direct phosphorylation of the RAPTOR1B regulatory associated protein of TOR, very similar to mTOR inhibition by AMPK [28*, 62]. Interestingly, there is also evidence of direct (parallel) SnRK1 regulation of typical TOR target processes. For example, while inactivation of TOR enables induction of autophagy, which then recycles cellular components to overcome starvation conditions [63], SnRK1 may also induce autophagy through direct phosphorylation and activation of the ATG1/13 kinase complex [64]. And while SnRK1 activation most likely represses general protein synthesis in part through TOR inhibition (massively saving energy), direct SnRK1 phosphorylation of eukaryotic translation initiation factor eIFiso4G1 was recently shown to mediate the selective translation of hypoxia-induced genes necessary for plants' tolerance to submergence [65]. Future more systematic analyses should further unravel the extent and nature of the SnRK1-TOR interactions in different tissues and conditions.

Several lines of evidence also indicate a close interaction between SnRK1 and SnRK2/ABA signaling (Figure 2). Consistently, overexpression of SnRK1 confers an ABA-hypersensitive phenotype [66]. SnRK2 signaling was recently also reported to be involved in maintaining the metabolic balance required for growth under non-stress conditions [67]. Both putative upstream and downstream molecular links have been found. SnRK1 was reported to phosphorylate group A bZIP TFs, involved in ABA signaling and controlling seed maturation and germination, *in vitro* [68]. Upstream, the PP2C phosphatases ABI1 and PP2CA, two known ABA/SnRK2 signaling repressors, were found to dephosphorylate the SnRK1 T-loop [14]. The plant-specific SnRK2 and SnRK3 (SNF1-related kinase 2 and 3) kinases belong to the same CDPK-SnRK superfamily, but outside the catalytic domain have limited similarity to the SnRK1 proteins [1]. In rice, the SnRK3 protein CIPK15 [calcineurin B–like (CBL)–interacting protein kinase15] was also identified as a putative SnRK1 upstream kinase, mediating seed α-amylases activation under oxygen deprivation, but direct phosphorylation was not reported [69].

Recently, an additional layer of interaction was uncovered with the reciprocal link between ABA/SnRK2 and TOR signaling (Figure 2). Under non-stress conditions, TOR phosphorylates and desensitizes the PYR/PYL (PYRABACTIN RESISTANCE/PYRLIKE) ABA receptors, disrupting interaction with (and inactivation of) the PP2C phosphatases and preventing phosphorylation-dependent SnRK2 activity [70*]. Upon stress, a small fraction of non-phosphorylated ABA receptors binds ABA, enabling SnRK2 de-repression, which then in turn also phosphorylates RAPTOR, resulting in dissociation and inactivation of the TOR complex [70*]. Whether and when TOR regulates SnRK1 activity, through PP2C activation or other mechanisms, requires further investigation.

Conclusions

Significant progress has been made in our understanding of SnRK1 biology, but many questions remain. Plants are typically exposed to (complex combinations of) biotic and abiotic stresses that affect plant metabolic status. However, all living organisms need to prioritize resources, and in the course of evolution a more general trade-off between growth and stress tolerance has been hardwired in genetic networks, notably the one comprising the antagonistic energy-sensing SnRK1 and TOR and the ABA signaling SnRK2 kinases in plants. Improving stress tolerance without yield penalty (or *vice versa*) by fine-tuning or conditional shifting of the balance will therefore require a more detailed insight in the underlying regulatory mechanisms and processes.

Highlights

. While SnRK1/SNF1/AMPK structure and function are well conserved, plants have modified SnRK1 regulation to better fit their unique physiology and lifestyle

. Rather than being activated by low energy stress, SnRK1 appears to be repressed by high energy signals, including sugar phosphates such as trehalose-6-P (T6P)

. The SnRK1 protein kinases exert their diverse effects through both direct phosphorylation of enzymes and regulatory proteins and extensive transcriptional reprogramming

. The group C/S1 bZIP transcription factors play a key role in this transcriptional response (with the group C bZIP63 as a direct SnRK1 target), regulating both growth and metabolism

. SnRK1 functions in an intricate network with the growth-stimulating and antagonistic TOR pathway and abscisic acid (ABA)-activated SnRK2 signaling

. Bypassing the trade-offs between growth and stress tolerance to improve crop yield will require more profound insight in the SnRK1-TOR-SnRK2 network

Acknowledgements

The authors would like to thank Tom Broeckx for help with the homology model of the SnRK1 complex and are grateful for the helpful reviewer comments. SnRK1 research in the Rolland lab is supported by the Fund for Scientific Research-Flanders (FWO, PhD fellowship to N.C.).

Figure captions

Figure 1. The SnRK1 heterotrimeric complex. Model of the SnRK1 complex based on individual subunit modelling on the 4RER crystal structure of phosphorylated (activated) AMPK [1]. The space-filling model (left) and cartoon representation (centre) give insight in the α, β and βγ subunit interactions with the β subunit functioning as the complex scaffold. The plant-specific hybrid βγ subunit contains four cystathionine β-synthase (CBS) domains (sensing nucleotide charge in AMPK) and an additional carbohydrate binding domain (βγCBM), apparently positioning close to the β subunit CBM [1]. The catalytic α subunit (right) harbors the active site, located in a catalytic cleft, and the T-loop with conserved threonine residue which needs to be phosphorylated for kinase activity. An α linker couples the ubiquitin-associated (UBA) domain to the far C-terminal domain (αCTD) involved in interaction with the other subunits as well as with upstream phosphatases [1].

Figure 2. Schematic overview of the SnRK-TOR signaling network, controlling the growth/stress tolerance balance. SnRK1 signaling saves and redirects energy used for active growth and development towards increased stress tolerance and survival under low energy conditions [1]. SnRK1 acts both by inhibition of the growth-stimulating and high energy-activated TOR kinase [57-59] and in a TOR-independent way, e.g. by transcriptional reprogramming mediated in part by the C/S1 group bZIP transcription factor (TF) network [13*,40-42]. SnRK1 signaling in addition cross-talks with abscisic acid (ABA)-induced SnRK2 stress signaling, upstream through their common regulation by the PP2C phosphatases [14], and possibly also downstream by targeting SnRK2-regulated A group bZIP TFs [68]. SnRK2 also represses TOR signaling by direct phosphorylation, resulting in TOR complex dissociation [70*]. In non-stress conditions, TOR activity in turn represses SnRK2 (and SnRK1) signaling via phosphorylation of the PYRABACTIN RESISTANCE1 (PYR1)/PYR1-LIKE (PYL) ABA-receptors and subsequent release of sequestered PP2C phosphatases [70*]. Rather than being activated by low energy stress, SnRK1 appears to be repressed by high energy signals, including sugar phosphates, such as trehalose-6-P (T6P), glucose-6-P (G6P) and glucose-1-P (G1P) [19,20]. This inhibition appears to be mediated by an intermediary proteinaceous factor [53], but T6P was recently also reported to directly bind to the SnRK1 catalytic subunit and inhibit its interaction with and phosphorylation by upstream SnAK/GRIK kinases [22*]. In addition, SnRK1 significantly autophosphorylates and also SnRK3 kinases may function upstream of SnRK1 [69]. Note that this

overview summarizes interactions reported in different species, tissues, or environmental condition, which are not necessarily occurring simultaneously. Full lines and dashed lines specify established and putative regulation, respectively.

References

[1] Broeckx T, Hulsmans S, Rolland F: The plant energy sensor: **Evolutionary conservation and divergence of SnRK1 structure, regulation, and function**. *Journal of Experimental Biology* 2016, **67**:6215-6252

[2] Hulsmans S, Rodriguez M, De Coninck B, Rolland F: **The SnRK1 energy sensor in plant biotic interactions**. *Trends in Plant Science* 2016, **21**:648-661

[3] Tsai AYL, Gazzarrini S: **Trehalose-6-phosphate and SnRK1 kinases in plant development and signaling: the emerging picture.** *Frontiers in Plant Science* 2014, **5**:119

[4] Baena-Gonzalez E, Hanson J: **Shaping plants development through the SnRK1-TOR metabolic regulators**. *Current Opinion in Plant Biology* 2017, **35**:152-157

[5] Hedbacker K, Carlson M: **SNF1/AMPK pathways in yeast**. *Frontiers in Bioscience* 2008, **13**:2408–2420

[6] Hardie DG, Schaffer BE, Brunet A: **AMPK: an energy-sensing pathway with multiple inputs and outputs**. *Trends in Cell Biology* 2016, **26:**190–201

[7] Garcia D, Shaw RJ: **AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance**. *Molecular Cell* 2016, **66**:789-800

[8] Emanuelle S, Doblin MS, Stapleton DI, Bacic A, Gooley PR: **Molecular insights into the enigmatic metabolic r**egulator, SnRK**1.** *Trends in Plant Science* 2016, 21, 341-353.

[9] Emanuelle S, Hossain MI, Moller IE, Henriette LP, van de Meene AML, Doblin MS, Koay A, Oakhill JS, Scott JW, Willats WGT *et al.*: **SnRK1 from *Arabidopsis thaliana* is an atypical AMPK.** *The Plant Journal* 2015, **82**:183–192 **Using complementary biochemical approaches, the authors clearly show that SnRK1 significantly differs from AMPK and SNF1 in its regulation and confirms that it uses the unique hybrid βγ protein as its canonical γ subunit.

[10] Ramon M, Ruelens P, Li Y, Sheen J, Geuten K, Rolland F: **The hybrid Four-CBS-Domain KINβγ subunit functions as the canonical subunit of the plant energy sensor SnRK1**. *The Plant Journal* 2013, **75**:11–25

[11] Maya-Bernal JL, Avila A, Ruiz-Gayosso A, Trejo-Fregoso R, Pulido N, Sosa-Peinoda A, Zuniga-Snachez E, Martinez-Barajas E, Rodriguez-Sotres R, Coello P: **Expression of recombinant SnRK1 in *E. coli.* Characterization of adenine nucleotide binding to the SnRK1.1/AKINβγ-β3 complex**. *Plant Science* 2017, **263**:116-125

[12] Emanuelle S, Doblin MS, Gooley PR, Gentry MS: **The UBA domain of SnRK1 promotes activation and maintains catalytic activity.** *Biochemical and Biophysical Research Communications* 2018, **497**:127-132

[13] Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J: **A central integrator of transcription networks in plant stress and energy signaling**. *Nature* 2007, **448**:938–942 *In this early study, transient expression of SnRK1α1 in leaf mesophyll protoplasts was used to explore the broad range of its transcriptional targets and identified the C/S1 group bZIP TFs as important mediators of SnRK1 signaling. Transgenic plants demonstrated the pivotal role of SnRK1 in the regulation of metabolism, growth and survival by stress, sugar and developmental signals.

[14] Rodrigues A, Adamo M, Crozet P, Margalha L, Confraria A, Martinho C, Elias A, Rabissi A, Lumbreras V, Gonzalez-Gutman M *et al*.: **ABI1 and PP2CA phosphatases are negative regulators of Snf1-related protein kinase1 signaling in *Arabidopsis*.** *The Plant Cell* 2013, **25:**3871–3884

[15] Shen W, Reyes MI, Hanley-Bowdoin L: ***Arabidopsis* protein kinases GRIK1 and GRIK2 specifically activate SnRK1 by phosphorylating its activation loop**. *Plant Physiology* 2009, **150**:996-1005

[16] Crozet P, Jammes F, Valot B, Ambard-Bretteville F, Nessler S, Hodges M, Vidal J, Thomas M: **Cross-phosphorylation between *Arabidopsis thaliana* sucrose nonfermenting 1-related protein kinase 1 (AtSnRK1) and its activating kinase (AtSnAK) determines their catalytic activities**. *Journal of Biological Chemistry* 2010, **285**: 12071–12077

[17] Glab N, Oury C, Guerinier T, Domenichini S, Crozet P, Thomas M, Vidal J, Hodges M: **The impact of *Arabidopsis thaliana* SNF1-related-kinase 1 (SnRK1)-activating kinase 1 (SnAK1) and SnAK2 on SnRK1 phosphorylation status: characterization of a SnAK double mutant**. *The Plant Journal* 2017, **89**:1031-1041

[18] Muraro D, Byrne HM, King JR, Bennett MJ: **Mathematical modelling plant signaling networks**. *Mathematical Modelling of Natural Phenomena* 2013, **8**:5-24

[19] Zhang Y, Primavesi LF, Jhurreea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ: **Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate**. *Plant Physiology* 2009, **149**:1860–1871

[20] Nunes C, Primavesi LF, Patel MK, Martinez-Barajas E, Powers SJ, Sagar R, Fevereiro PS, Davis BG, Paul MJ: **Inhibition of SnRK1 by metabolites: tissue-dependent effects and cooperative inhibition by glucose 1-phosphate in combination with trehalose 6-phosphate**. *Plant Physiology and Biochemistry* 2013, **63**:89–98

[21] Lunn JE, Delorge I, Figueroa CM, Van Dijck P, Stitt M: **Trehalose metabolism in plants**. *The Plant Journal* 2014, **79**:544–567

[22] Zhai Z, Keereetaweep J, Liu H, Feil R, Lunn JE, Shanklin J: **Trehalose-6-phosphate positively regulates fatty acid synthesis by stabilizing WRINKLED1**. *The Plant Cell* 2018, **30**:2616-2627 *How T6P regulates SnRK1 activity arguably is one of the most intriguing and

challenging questions in the field. This study now reports the surprising finding that T6P may directly bind to the SnRK1α subunit (using fluorescence-based microscale thermophoresis) and interfere with upstream kinase interaction and T-loop phosphorylation

[23] Nuccio ML, Wu J, Mowers R, Zhou HP, Mehji M, Primavesi LF, Paul MJ, Chen X, Gao Y, Haque E *et al*.: **Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions.** *Nature Biotechnology* 2015, **33**:862-869

[24] Griffiths CA, Sagar R, Geng Y, Primavesi LF, Patel MK, Passarelli MK, Gilmore IS, Steven RT, Bunch J, Paul MJ *et al*.: **Chemical intervention in plant sugar signaling increases yield and resilience**. *Nature* 2016, **540**: 574-578

[25] Avila-Castaneda A, Gutierrez-Granados N, Ruiz-Gayosso A, Sosa-Peinado A, Martinez-Barajas E, Coello P: **Structural and functional basis for starch binding in the SnRK1 subunits AKINβ2 and AKINβγ**. *Frontiers in Plant Science* 2014, **5**:199

[26] Ruiz-Gayosso, A, Rodrigues-Sotres R, Martinez-Barajas E, Coello P: **A role for the carbohydrate-binding module (CBM) in regulatory SnRK1 subunits: the effect of maltose on SnRK1 activity**. *The Plant Journal* 2018, **96**:163-175

[27] Gao XQ, Liu CZ, Li DD, Zhao TT, Li F, Jia XN, Zhao XY, Zhang XS: **The *Arabidopsis* KINβγ subunit of the SnRK1 complex regulates pollen hydration on the stigma by mediating the level of reactive oxygen species in pollen**. *PLoS Genetics* 2016, **12**:e1006228

[28] Nukarinen E, Nagele T, Pedrotti L, Wurzinger B, Mair A, Landgraf R, Bornke F, Hanson J, Teige M, Baena-Gonzalez E *et al*.: **Quantitative phosphoproteomics reveals the role of the AMPK plant ortholog SnRK1 as a metabolic master regulator under energy deprivation**. *Science Reports* 2016, **6**:31697. *The authors used an extended darkness condition in combination with transgenic SnRK1α over-expression and (inducible) knockdown for a combined *in vivo* phosphoproteome, proteome and metabolome analysis, identifying many new (putative) SnRK1 targets, including the major TOR target RPS6. They provide specific molecular evidence for a direct link with TOR signalling by demonstrating *in vivo* interaction between SnRK1□□ and RAPTOR1B and phosphorylation of RAPTOR1B by SnRK1□1.

[29] Blanco NE, Liebsch D, Diaz MG, Strand A, Whelan J: **Dual and dynamic localisation of *Arabidopsis thaliana* SnRK1.1.** *Journal of Experimental Biology* 2019, doi: 10.1093/jxb/erz023

[30] Lin CR, Lee KW, Chen CY, Hong YF, Chen JL, Lu CA, Chen KT, Ho THD, Yu SM: **SnRK1A-interacting negative regulators modulate the nutrient starvation signaling sensor SnRK1 in source-sink communication in cereal seedlings under abiotic stress**. *The Plant Cell* 2014, **26**:808-827

[31] Lin SC and Hardie DG: **AMPK: Sensing glucose as well as cellular energy status**. *Cell Metabolism* 2018, **27**:299-313

[32] Crozet P, Margalha L, Butowt R, Fernandes N, Elias A, Orosa B, Tomanov K, Teige M,

Bachmair A, Sadanandom A *et al*.: **SUMOylation represses SnRK1 signaling in *Arabidopsis***. *The Plant Journal* 2016, **85**:120–133

[33] Nietzsche M, Schieβl I, Bornke F: **The complex becomes more complex: protein-protein interactions of SnRK1 with DUF581 family proteins provide a framework for cell- and stimulus type-specific SnRK1 signaling in plants**. *Frontiers in Plant Science* 2014, **5**:54

[34] Nietzsche M, Landgraf R, Tohge T, Bornke F: **A protein–protein interaction network linking the energy-sensor kinase SnRK1 to multiple signaling pathways in *Arabidopsis thaliana***. *Current Plant Biology* 2016, **5**:36-44

[35] Jamsheer M, Sharma M, Singh D, Mannully CT, Jindal S, Shukla BN, Laxmi A: **FCS-like zinc finger 6 and 10 repress SnRK1 signaling in *Arabidopsis***. *The Plant Journal* 2018, **94**:232-245

[36] Wurzinger B, Mair A, Fischer-Schrader K, Nukarinen E, Roustan V, Weckwerth W, Teige M: **Redox state-dependent modulation of plant SnRK1 kinase activity differs from AMPK regulation in animals**. *FEBS Letters* 2017, **591**:3625-3636

[37] Robertlee J, Kobayashi K, Suzuki M, Muranaka T: **AKIN10, a representative *Arabidopsis* SNF1-related protein kinase 1 (SnRK1), phosphorylates and downregulates plant HMG-CoA reductase**. *FEBS Letters* 2017, **519**:1159-1166

[38] Cho HY, Wen TN, Wang YT, Shih MC: **Quantitative phosphoproteomics of protein kinase SnRK1 regulated protein phosphorylation in *Arabidopsis* under submergence**. *Journal of Experimental Botany* 2016, **67**:2745–2760

[39] Cho HY, Hong JW, Kim EC, Yoo SD: **Regulatory functions of SnRK1 in stress-responsive gene expression and in plant growth and development**. *Plant Physiology* 2012, **158**:1955-1964

[40] Droge-Laser W, Weiste C: **The C/S1 bZIP network: a regulatory hub orchestrating plant energy homeostasis**. *Trends in Plant Science* 2018, **23**:422-433

[41] Mair A, Pedrotti L, Wurzinger B, Anrather D, Simeunovic A, Weiste C, Valerio C, Dietrich K, Kirchler T, Nagele T *et al*.: **SnRK1-triggered switch of bZIP63 dimerization mediates the low-energy response in plants**. *eLife* 2015, **4**:e05828 *This work corroborates the C-group bZIP63 TF as a direct SnRK1 target, with evidence of both *in vitro* and *in vivo* phosphorylation of three conserved serine residues. The authors also show that phosphorylation alters the heterodimerization ability of bZIP63 to induce gene expression during energy starvation.

[42] Pedrotti L, Weiste C, Nagele T, Wolf E, Lorenzin F, Dietrich K, Mair A, Weckwerth W, Teige M, Baena-Gonzalez E, Droge-Laser W: **Snf1-RELATED KINASE1-controlled C/S1-bZIP signaling activates alternative mitochondrial metabolic pathways to ensure plant survival in extended darkness**. *The Plant Cell* 2018, **30**:495-509 *This study reports the induction of branched-chain amino acid catabolism which feeds into an alternative mitochondrial respiratory pathway during carbon starvation. The authors also provide evidence for the formation of a C1/S-SnRK1 complex that interacts with the histone acetylation machinery to remodel chromatin and facilitate transcription.

[43] Weiste C, Pedrotti L, Selvanayagam J, Muralidhara P, Froschel C, Novak O, Ljung K, Hanson J, Droge-Laser W: **The *Arabidopsis* bZIP11 transcription factor links low-energy signaling to auxin-mediated control of primary root growth**. *PLoS Genetics* 2017, **13**:e1006607 *This study reports how the group S bZIP11 TF directly activates the IAA3/SHY2 repressor promoter to negatively regulate auxin signalling and PIN auxin efflux carrier expression, providing a straightforward and effective mechanism to limit root growth in carbon starvation conditions.

[44] Flis A, Sulpice R, Seaton DD, Ivakov AA, Liput M, Abel C, Millar AJ, Stitt M: **Photoperiod-dependent changes in the phase of the core clock transcripts and global transcriptional outputs at dawn and dusk in *Arabidopsis***. *Plant, Cell and Environment* 2016, **39**:1955-1981

[45] Shin J, Sanchez-Villareal A, Davis AM, Du SX, Berendzen KW, Konec C, Ding Z, Li C, Davis SJ: **The metabolic sensor AKIN10 modulates the *Arabidopsis* circadian clock in a light-dependent manner**. *Plant, Cell and Environment* 2017, **40**:997-1008

[46] Sanchez-Villarreal A, Davis AM, Davis SJ: **AKIN10 activity as a cellular link between metabolism and circadian-clock entrainment in *Arabidopsis thaliana***. *Plant Signalling and Behavior* 2018, **13**:e1411448

[47] Frank A, Matiolli CC, Viana AJC, Heam TJ, Kusakina J, Belbin FE, Newman DW, Yochikawa A, Cano-Ramirez DL, Chembath A *et al*.: **Circadian entrainment in *Arabidopsis* by the sugar-responsive transcription factor bZIP63**. *Current Biology Report* 2018, **28**:2597-2606 * This study identified the bZIP63 TF as a component in sugar-mediated regulation of the circadian oscillator, directly binding to the PRR7 promoter, downstream of T6P and SnRK1. It illustrates how sugar and energy supplies not only reset the clock through indirect metabolic effects, but apparently also by direct SnRK1 signaling

[48] Wiese A, Elzinga N, Wobbes B, Smeekens S**: A conserved upstream open reading frame mediates sucrose-induced repression of translation**. *The Plant Cell* 2004, **16:**1717–1729

[49] Jeong EY, Seo PJ, Woo JC, Park CM: **AKIN10 delays flowering by inactivating IDD8 transcription factor through protein phosphorylation in *Arabidopsis***. *BMC Plant Biology* 2015, **15**:110

[50] Tsai AYL, Gazzarrini S: **AKIN10 and FUSCA3 interact to control lateral organ development and phase transitions in *Arabidopsis***. *The Plant Journal* 2012, **69**:809-821

[51] Chan A, Carianopol C, Tsai AY, Varatharajah K, Chiu RS, Gazzarrini S: **SnRK1 phosphorylation of FUSCA3 positively regulates embryogenesis, seed yield, and plant growth at high temperature in *Arabidopsis***. *Journal of Experimental Botany* 2017, **68**:4219-4231

[52] Zhai Z, Liu H, Shanklin J: **Phosphorylation of WRINKLED1 by KIN10 results in its proteasomal degradation, providing a link between energy homeostasis and lipid biosynthesis**. *The Plant Cell* 2017, **29**:871-889

[53] Kim GD, Cho YH, Yoo SD: **Regulatory functions of cellular energy sensor SNF1-related**

kinase1 for leaf senescence delay through ETHYLENE-INSENSITIVE3 repression. *Nature Scientific Reports* 2017, **7**:3193

[54] Nietzsche M, Guerra T, Alseekh S, Wiermer M, Sonnewald S, Fernie AR, Bomke F: **STOREKEEPER RELATED1/G-element binding protein (STKR1) interacts with protein kinase SnRK1**. *Plant Physiology* 2018, **176**:1773-1792

[55] Jamsheer KM, Laxmi A: **Expression of *Arabidopsis* FCS-Like Zinc finger genes is differentially regulated by sugars, cellular energy level, and abiotic stress.** *Frontiers in Plant Science* 2015, 6:746

[56] Confraria A, Martinho C, Elias A, Rubio-Somoza I, Baena-Gonzalez E**: miRNAs mediate SnRK1-dependent energy signaling in *Arabidopsis***. *Frontiers in Plant Science* 2013, **4:**197

[57] Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C**: TOR signaling and nutrient sensing**. *Annual Review of Plant Biology* 2016, **67:**261–285.

[58] Shi L, Wu Y, Sheen J: **TOR signaling in plants: conservation and innovation**. *Development* 2018, 145:dev160887

[59] Wu Y, Shi L, Li L, Fu L, Liu Y, Xiong Y, Sheen J: **Integration of nutrient, energy, light and hormone signalling via TOR in plants**. *Journal of Experimental Botany* 2019, Epub ahead of print. doi: 10.1093/jxb/erz028

[60] Xiong Y, McCormack M, Li L, Hall Q, Xiang C, Sheen J: **Glucose–TOR signaling reprograms the transcriptome and activates meristems**. *Nature* 2013, **496:**181–186

[61] Li L, Sheen J: **Dynamic and diverse sugar signaling**. *Current Opinion in Plant Biology* 2016, **33**:116-125

[62] Van Leene J, Han C, Gadeyne A, Eeckhout D, Matthijs C, Cannoot B, De Winne N, Persiau G, Van De Slijke E, Van de Cotte B *et al.*: **Capturing the phosphorylation and protein interaction landscape of the plant TOR kinase.** *Nature Plants* 2019, **5**:316-327

[63] Soto-Burgos J, Bassham DC: **SnRK1 activates autophagy via the TOR signaling pathway in *Arabidopsis thaliana***. *PLoS ONE* 2017, **12**:e0182591

[64] Chen L, Su ZZ, Huang L, Xia FN, Qi H, Xie LJ, Xiao S, Chen QF: **The AMP-activated protein kinase KIN10 is involved in the regulation of autophagy in *Arabidopsis***. *Frontiers in Plant Science* 2017, **8**:1201

[65] Cho H-Y, Jade Lu M-Y, Shih M-C: **The SnRK1-eIFiso4G1 signaling relay regulates the translation of specific mRNAs in *Arabidopsis* under submergence**. *New Phytologist* 2018, doi: 10.1111/nph.15589

[66] Jossier M, Bouly JP, Meimoun P, Arjmand A, Lessard P, Hawley S, Grahame Hardie D, Thomas M: **SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signaling in *Arabidopsis thaliana*.** *The Plant Journal* 2009, **59**:316–328

[67] Yoshida T, Obata T, Feil R, Lun JE, Fujita Y, Yamaguchi-Shinozaki K, Fernie AR: **The role of abscisic acid signalling in maintaining the metabolic balance required for *Arabidopsis***

growth under nonstress conditions. *The Plant Cell* 2019, **31**:84-105

[68] Bitrian M, Roodbarkelari F, Horvath M, Koncz C: **BAC- recombineering for studying plant gene regulation: developmental control and cellular localization of SnRK1 kinase subunits**. *The Plant Journal* 2011, **65:**829–842.

[69] Lee KW, Chen PW, Lu CA, Chen S, Ho TH, Yu SM: **Coordinated responses to oxygen and sugar deficiency allow rice seedlings to tolerate flooding.** *Science Signaling* 2009, **2**:ra61.

[70] Wang P, Zhao Y, Li Z, Hsu CC, Liu X, Fu L, Hou YJ, Du Y, Xie S, Zhang C *et al*.: **Reciprocal regulation of the TOR kinase and ABA receptor balances growth and stress response**. *Molecular Cell* 2018, **69**:100-112 * This study convincingly shows that that reciprocal (feedback) regulation of the TOR kinase and SnRK2-mediated ABA signaling contributes to balancing growth and stress responses. While the growth-promoting TOR kinase phosphorylates the (PYL) ABA receptors to prevent SnRK2 activation of the stress response in non-stress conditions, ABA-activated SnRK2s phosphorylate and inactivate the TOR complex.