Shaping plant development through the SnRK1-TOR metabolic regulators

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Highlights:

- Sugars serve as nutrients and as regulatory signals
- SnRK1 and TOR control metabolism and growth oppositely in response to sugars
- Manipulation of SnRK1 and TOR results in highly diverse developmental phenotypes
- Sugar signals shape plant development *via* SnRK1 and TOR

SnRK1 (Snf1-related protein kinase 1) and TOR (target of rapamycin) are evolutionarily conserved protein kinases that lie at the heart of energy sensing, playing central and antagonistic roles in the regulation of metabolism and gene expression. Increasing evidence links these metabolic regulators to numerous aspects of plant development, from germination to flowering and senescence. This prompts the hypothesis that SnRK1 and TOR modify developmental programs according to the metabolic status to adjust plant growth to a specific environment. The aim of this review is to provide support to this hypothesis and to incentivize further studies on this topic by summarizing the work that establishes a genetic connection between SnRK1-TOR and plant development.

Introduction

As autotrophic organisms, plants produce sugars in mature photosynthetic leaves (source organs) to support storage and growth in sink tissues (roots, fruits and young leaves). Sugars drive growth by serving both as metabolic substrates and as signals that tightly interact with hormonal, environmental, and other metabolic cues to coordinate cell growth in specific tissues with storage and nutrient remobilization [1]. In doing so, sugars have been linked to stress responses and growth control, but an increasing amount of studies implicate sugar signals also in developmental decisions such as germination, flowering, and senescence [2,3].

Two evolutionarily conserved protein kinases are at the heart of sugar sensing and plant energy management, the Snf1-related protein kinase1 (SnRK1; AMPK in mammals and Snf1 in yeast) and the Target of Rapamycin (TOR) protein kinase, which play central and antagonistic roles [1] (Fig. 1). The individual components of the SnRK1 and TOR pathways vary among eukaryotes, but the overall structure is remarkably similar [4,5]. SnRK1 is activated in response to declining energy supplies (e.g., during stress), triggering the activation of catabolism and the repression of energy-consuming anabolic processes and growth. SnRK1 signaling during stress is blocked by sugars [6,7], possibly in the form of distinct sugar phosphates such as trehalose-6-phosphate (T6P) [8-10], although the connection between T6P and SnRK1 is still a matter of debate [11]. Conversely, TOR promotes growth and biosynthetic processes such as ribosome biogenesis and protein synthesis in response to energy availability [12], e.g. in the form of glucose [13]. However, how exactly sugar availability affects SnRK1 and TOR pathway activity is not known.

Both kinases exert their function through wide-ranging transcriptional reprogramming and metabolic readjustments. The transcriptional profiles associated with SnRK1 and TOR activity are largely opposite, supporting their globally antagonistic roles [1,6,12-14]. Furthermore, SnRK1 phosphorylates key enzymes involved in nitrogen and carbon metabolism [15,16], and SnRK1 manipulation has major consequences for primary metabolism, partly *via* specific bZIP transcription factors [6,17-19]. In turn, decreased TOR activity causes accumulation of sugars [20] and amino acids [21].

Mounting evidence also links SnRK1-TOR signaling to developmental processes, possibly as a result from altered metabolism and carbon allocation and/or from more specific interactions with developmental pathways. In this review we summarize the rapidly increasing literature connecting SnRK1 and TOR to multiple developmental processes to argue that metabolic signaling through the SnRK1-TOR axis is crucial for adjusting plant development to the environment. Due to space limitations, we focus on studies based on the genetic or chemical manipulation of the SnRK1 and TOR kinase complexes.

Seed development and germination

The SnRK1-TOR signaling network impacts plant growth from the very early stages of development. In Arabidopsis, single mutations in the SnRK1α catalytic subunit genes (Fig. 1) do not cause obvious phenotypes under standard growth conditions [6]. However, double *snrk1α1/snrk1α2* knockout mutants have not been successfully generated, suggesting transmission defects (see section on reproduction) and/or embryo lethality. In pea, postembryonic silencing of *SnRK1α* through a seed storage protein promoter results in defective cotyledon development [22] and seed maturation, including reduced accumulation of protein reserves, impaired desiccation tolerance, and precocious germination [23]. These effects are accompanied by altered expression of genes related to cell proliferation and differentiation, leaf polarity, and seed maturation, such as *FUSCA3* and *ABI3*. Furthermore, SnRK1 repression reduces the accumulation of cytokinin and ABA, revealing a connection between SnRK1 and the auxin/cytokinin and ABA pathways [22]. Consistent with this, SnRK1α1 overexpression in Arabidopsis causes delayed germination [24]. SnRK1 phosphorylates the

FUSCA3 transcription factor *in vitro*, and FUSCA3 degradation is delayed in cell extracts from *35S::SnRK1α1* plants. Furthermore, *35S::SnRK1α1 fusca3-3* double mutants display precocious germination and desiccation intolerance (similar to *fusca3-3*), suggesting that SnRK1 delays germination by stabilizing FUSCA3 [24]. Unexpectedly, loss of function of SnRK1A, the closest homolog of Arabidopsis SnRK1α1 in rice, also results in delayed germination and seedling development [25], indicating differences between dicots and monocots or compensatory effects from the remaining rice *SnRK1α* genes.

In the case of TOR, knockout *tor* embryos arrest early, during the dermatogen stage [26], and endosperm development is abrogated due to impaired cell division and a lack of cellularization. These phenotypes are in agreement with the expression of TOR in developing seed tissues and underscore the essentiality of this kinase for cell proliferation and multicellularity. Embryo lethality has also been shown for mutants in the *RAPTOR* gene [27]. In addition, TOR appears to promote growth at later stages of development; for example, overexpression of the TOR downstream effector TAP46 results in larger seeds with increased longevity [20,28]. Furthermore, when TOR activity is inhibited chemically or through mutations in the *LST8/RAPTOR* genes, germination and early seedling development are negatively affected, which may partially be due to changes in ABA levels [29] and signaling [30]. The well-established role of TOR in promoting translation has also been suggested to be important for its positive effect on seed germination [31].

Seedling establishment and vegetative growth

After germination, the developing seedling grows mostly through cell elongation using seed reserves and this is followed by the initiation of meristem activity and cell division. This switch to proliferative growth is likely triggered by the availability of photosynthates, as exogenous sugar provision is sufficient to support growth and organ formation [32]. Post-germination growth requires TOR activity. Firstly, mutants of the TOR complex show growth retardation [20] that can be attributed to a direct positive effect of TOR on cell division [33], translation [12], photosynthesis [34], and sugar-induced cell elongation [35]. Secondly, the specific TOR inhibitor AZD-8055 blocks seedling growth induced by sugars or light [30,36]. Thirdly, TOR reactivates the root meristem after germination in a sugar (glucose)-dependent manner [13]. If unfavorable conditions are encountered following germination, developmental arrest is induced by ABA [37], which can be mimicked by high exogenous sugar supply [38]. Overexpression of SnRK1α1 or SnRK1β1 causes glucose and ABA hypersensitivity during early seedling development [39,40] leading to defective cotyledon expansion and true leaf formation. Furthermore, a mutant lacking the RNA splicing factor SR45 is hypersensitive to sugar, partly due to SnRK1α1 over-accumulation [41]. SnRK1α1 overexpression was further associated with defective cotyledon number and morphology in a FUSCA3-dependent manner [24].

Thus far, full SnRK1α knockouts have only been reported in the moss *Physcomitrella patens* [42]. At the protonemal stage wild-type colonies consist primarily of photosynthetically active chloronemal filaments, while double *snf1a snf1b* mutants generate mostly caulonemal filaments with a lower photosynthetic capacity. During the subsequent gametophore stage, a lack of SnRK1 results in a higher rhizoid/stem ratio, consistent with a hyposensitivity to cytokinin and auxin hypersensitivity. Double *snf1a snf1b* mutants are only viable when grown under continuous light or with exogenous sugar, likely because of their inability to synthesize starch. In contrast, transient SnRK1α silencing in Arabidopsis *via* virus-induced gene silencing (VIGS) causes growth arrest that cannot be rescued by sugar or constant light, indicating fundamental differences from lower plants and defects in growth regulation [6]. Supporting this, silencing of a potato SnRK1β subunit (*StubGAL83*) results in reduced root growth partly due to decreased cell size [43]. In the case of TOR, overexpression of the kinase domain causes abnormal leaf lamina development and twisted and crocked siliques [44], probably as a consequence of localized deregulation of growth.

Flowering and plant architecture

An increasing number of studies demonstrate the relevance of the SnRK1 and TOR pathways for flowering. Arabidopsis plants overexpressing the SnRK1α1 subunit are late flowering under long days (16:8) [24,45], and, likewise the delay in germination, this can be partially rescued by the *fusca3-3* mutation, indicating an intriguing connection between SnRK1 and FUSCA3 in the general regulation of phase transitions [24]. The regulatory sugar T6P regulates flowering *via* the photoperiod and age pathways [46], but whether this is related to its inhibitory effect on SnRK1 is unknown. Flowering is accelerated under low nitrogen conditions in a cryptochrome 1 (CRY1)-dependent manner and this may involve the repression of SnRK1 activity to allow CRY1 accumulation [47].

Flowering can also be initiated in the dark by prolonged sugar treatment [32], and the activation of stem cells in this case requires TOR function [36]. Similarly, the late-flowering phenotype of *lst8* and *raptor* mutants [21,48] suggests that TOR promotes flowering also under normal light conditions.
SnRK1 affects reproduction beyond the commitment to flower. Weaker VIGS-mediated SnRK1α silencing allows the formation of inflorescences, but the resulting flower buds are unviable [6]. Pollen development seems to be particularly sensitive to SnRK1 manipulation, and the silencing of *SnRK1α* in pollen results in smaller, wrinkled grains and male sterility [23,49,50]. Depletion of the SnRK1βγ subunit leads to defective hydration and germination of pollen grains on the stigma, which may be explained by defective mitochondria and peroxisome biogenesis and altered ROS signaling [50]. Finally, *raptor1B and lst8* mutants exhibit increased shoot branching [21,48], suggesting TOR as a player in the increasing connections between sugars and the regulation of apical dominance and axillary meristem activation during branching [51].

Senescence
SnRK1-TOR signaling pathways also have a strong impact on natural and dark-induced senescence, during which nutrients are remobilized to young developing organs and/or seeds. Plants overexpressing kinase-dead SnRK1α1 exhibit accelerated natural senescence [52], while plants overexpressing WT SnRK1α1 have the opposite phenotype [6,52]. Surprisingly, knockout mutants of the SnRK1 target transcription factor bZIP63 display delayed dark-induced senescence [18]. This is rescued by the WT bZIP63 but not by a bZIP63 variant that cannot be phosphorylated by SnRK1 [18], altogether suggesting SnRK1 represses bZIP63 in the context of senescence. In contrast to other processes, TOR and SnRK1 affect senescence similarly. TOR inactivation causes early senescence, while TOR overexpression delays the process [20]. Overexpression of the TOR kinase domain on the other hand, causes accelerated senescence, potentially by exerting a dominant negative effect [44]. It is plausible that the effect of TOR on senescence occurs *via* the repression of autophagy. Firstly, the autophagic degradation of cellular constituents plays an important role during senescence [53]. Secondly, in yeast and mammals, autophagy is regulated by the TOR and SnRK1 homologs in a negative and positive manner, respectively [54], and the autophagy machinery is largely conserved in plants [55]. Supporting a conserved function of TOR and SnRK1 on autophagy, both kinases have been shown to transcriptionally regulate autophagy-related genes [6,55].

Signal integration
In mammalian cells, TOR activity is inhibited by AMPK via the TSC1/TSC2/Rheb pathway [56,57] and by phosphorylation of the TOR complex protein RAPTOR [58]. In turn, the TOR downstream kinase S6K was shown to directly inactivate mammalian AMPK by phosphorylation [59]. While the absence of several components of the mammalian AMPK-TOR axis in plants suggests fundamental differences compared with metazoans, experimental evidence and sequence conservation indicates that the general structure of these signaling pathways is conserved [5]. A global analysis of the transcriptomic changes that occur upon manipulation of the SnRK1-TOR network revealed that the opposing functions of the two kinases hold true for plants [6,14]. Accordingly, SnRK1 can interact with RAPTOR *in vivo* [60], although this interaction may require the scaffolding action of the plant-specific DUF581-19/MARD1 protein, as shown in yeast two-hybrid assays [61]. Global phosphoproteomic analyses of plants with altered SnRK1 activity have also revealed altered phosphorylation levels of classical TOR targets such as RPS6 [60] and translation initiation factors [62]. The plant SnRK1-TOR system is heavily intertwined also with hormone signaling pathways. The SnRK1 pathway is moderated by ABA signaling [63], and downstream effectors regulate auxin-controlled processes [64]. TOR is also modulated by auxin signaling and regulates the expression of auxin signaling components by facilitating the translation of uORF-containing mRNAs [65]. In addition, TOR promotes brassinosteroid signaling by stabilizing the BZR1 transcription factor [35]. Further downstream, TOR signaling induces the expression of genes involved in ABA, ethylene, salicylic acid and jasmonate signaling while repressing genes involved in the auxin, cytokinin, brassinosteroid, and gibberellin pathways [14].

Future challenges
The centrality of SnRK1 and TOR is manifested as the collapse of plant growth when these components are inactivated. Their manipulation also results in numerous alterations in stress tolerance, and in developmental processes, such as phase transitions, plant architecture, reproduction, and senescence, but whether all of these processes are under the direct control of SnRK1 and TOR or they are pleiotropic long-term cumulative effects derived from altered metabolism remains to be determined. The identification of direct targets (enzymes, transcription factors, others) that connect SnRK1-TOR to distinct cellular or whole-plant processes is a first step in understanding

how these central kinases affect development. Increasing molecular connections have been established between the plant carbon status and hormone synthesis, transport and/or signaling [66] but further work is required to assess the involvement of the SnRK1-TOR axis in these processes. Key to the progress of this field is the development of better genetic and chemical tools that allow the manipulation of these components in an inducible or tissue-specific manner. Although specific chemical inhibitors are available for TOR, the same is not true for the SnRK1 pathway. A more precise manipulation of these pathways may also be facilitated by a deeper understanding of SnRK1 and TOR regulation. Although SnRK1 and TOR signaling are clearly dependent on the energy/sugar status, the molecular details of signal initiation and termination remain mostly unknown. Despite these complications, increasing interest in SnRK1-TOR signaling has attracted new researchers who are rapidly advancing this field with novel perspectives and technologies. We expect that more molecular connections between metabolism and development will be unveiled and that SnRK1 and TOR signaling will be attributed important roles in developmental programs.

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Figure 1. SnRK1 and TOR are protein kinase complexes that regulate in an antagonistic manner growth and development in response to sugar signals.
SnRK1 is a heterotrimeric protein kinase complex composed of an α-catalytic subunit and regulatory β and βγ subunits. In plants most subunits are encoded by several genes (*SnRK1α1/α2*, *SnRK1β1/β2/β3*, and *SnRK1βγ* in Arabidopsis), resulting in multiple potential complexes [67,68]. The TOR complex, in turn, consists of the TOR kinase and the RAPTOR and LST8 proteins, which is equivalent to the mammalian TORC1 complex. The TORC2 complex, conserved in animals and yeast, is absent from plants [5]. In response to as yet unidentified energy and nutrient signals, the SnRK1 and TOR kinases regulate metabolism and gene expression in a largely antagonistic manner. This results in the promotion of growth and developmental progression by TOR and the suppression of these processes by SnRK1.

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