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REVIEW PAPER

TOR kinase, a GPS in the complex nutrient and hormonal signaling networks to guide plant growth and development

Yanyan Meng[1,2,†], Nan Zhang[1,2,†], Jiatian Li[1,2], Xuehong Shen[1,2], Jen Sheen[3] and Yan Xiong[1,2,*]

[1] College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[2] Haixia Institute of Science and Technology, Plant Synthetic Biology Center, Fujian Agriculture and Forestry University, Fuzhou 350002, China
[3] Department of Molecular Biology and Centre for Computational and Integrative Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA 02114, USA

† These authors contributed equally to this work.
* Correspondence: yanxiong@fafu.edu.cn

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Abstract

To survive and sustain growth, sessile plants have developed sophisticated internal signalling networks that respond to various external and internal cues. Despite the central roles of nutrient and hormone signaling in plant growth and development, how hormone-driven processes coordinate with metabolic status remains largely enigmatic. Target of rapamycin (TOR) kinase is an evolutionarily conserved master regulator that integrates energy, nutrients, growth factors, hormones, and stress signals to promote growth in all eukaryotes. Inspired by recent comprehensive systems, chemical, genetic, and genomic studies on TOR in plants, this review discusses a potential role of TOR as a 'global positioning system' that directs plant growth and developmental programs both temporally and spatially by integrating dynamic information in the complex nutrient and hormonal signaling networks. We further evaluate and depict the possible functional and mechanistic models for how a single protein kinase, TOR, is able to recognize, integrate, and even distinguish a plethora of positive and negative input signals to execute appropriate and distinct downstream biological processes via multiple partners and effectors.

Keywords: Auxin, circadian clock, glucose, hormone sensing, nitrogen, nutrient sensing, target of rapamycin.

Introduction

Nutrient and hormonal signaling constitute two of the most ancient and fundamental regulatory networks that control the growth and development of plants and animals. Unlike animals, plants lack neuronal systems and rely mostly on mobile signals, such as hormones, sugars, peptides, proteins, and RNAs, for cell-to-cell and organ communication (Chaiwanon *et al.*, 2016). Not surprisingly, sessile plants have developed highly dynamic and sophisticated signaling networks to coordinate their nutrient and energy status with the hormone-driven processes. It has been proved that these intertwined signaling networks are connected by multiple signaling hubs and transducers (Chaiwanon *et al.*, 2016).

In recent years, different putative signaling hubs, including transcription factors (TFs), enzymes, receptors, and protein kinases, have been identified based on their abilities to respond to diverse nutrients (e.g. sugar, nitrogen) and hormone signals at either the transcriptional, translational, or post-translational level (Moore *et al.*, 2003; Sairanen *et al.*, 2012; Li *et al.*, 2014; K. Li *et al.*, 2015; Zentella *et al.*, 2016; Zhang *et al.*, 2016).

Target of rapamycin (TOR) is an atypical Ser/Thr protein kinase belonging to the phosphatidylinositol 3-kinase-related kinase (PIKK) family (Y. Wu *et al.*, 2019). It was originally identified in *Saccharomyces cerevisiae* by screening mutants insensitive to treatment with the antibiotic rapamycin, which was serendipitously isolated from the bacterium *Streptomyces hygroscopicus* in a soil sample collected on Easter Island (Sehgal *et al.*, 1975; Heitman *et al.*, 1991; Kahan, 2003). Since its discovery, extensive research has demonstrated an essential role of TOR as a central hub that modulates nutrient availability, cellular energy status, and hormone and stress signaling to drive cellular and organismal growth in all eukaryotes (Brunkard, 2020; Liu and Sabatini, 2020). Understanding of the mechanisms and functions of TOR signaling in plants has progressed immensely in the past decade. In plants, TOR senses not only the conserved upstream signals, such as glucose, amino acids (AAs), nucleotides and energy, but also plant-specific signals, such as light, phytohormones, inorganic nitrate, ammonium, phosphorus and sulfate, which may be caused by specific evolutionary adaptations to plants' unique immobile and autotrophic lifestyles. TOR tightly controls various downstream processes, including the cell cycle, nutrient assimilation and transportation, primary and secondary metabolism, transcription, translation, the circadian clock, and autophagy (Li *et al.*, 2021). Indeed, *TOR* mRNA and TOR protein are expressed in almost all organs, tissues, and cell types, according to extensive transcriptomic and proteomic studies (Brunkard, 2020). TOR participates in various stages of plant growth and development from embryogenesis, heterotrophic seedling growth, and photoautotrophic seedling establishment to root and shoot meristem activation, root hair elongation, leaf expansion and vegetative growth, flowering, and senescence (Shi *et al.*, 2018; Quilichini *et al.*, 2019).

Recent studies have started to reveal a role of TOR as a central hub integrating complex nutrient and hormonal signaling pathways to orchestrate multiple downstream processes (Brunkard, 2020; Li *et al.*, 2021; Liu and Xiong, 2021). These different upstream signaling pathways may converge on TOR to perform the same or similar downstream functions, or individually influence TOR to conduct completely different functions. In this review, we discuss the mechanistic possibilities of how a single central protein kinase, TOR, is able to recognize and integrate or distinguish diverse upstream signals to modulate a myriad of cellular activities via multiple partners and effectors—essentially, acting like a global positioning system (GPS) to spatiotemporally integrate dynamic information in the complex nutrient and hormonal signaling networks to precisely direct the appropriate plant developmental and physiological responses.

TOR guides temporal plant growth and development by integrating upstream signals

TOR signaling has been found to orchestrate plant growth and development throughout the life cycle, from embryogenesis and seedling growth to vegetative and reproductive growth, by modulating multiple downstream cellular processes (Liu and Xiong, 2021). Here, we mainly discuss how TOR senses and integrates diverse internal and external upstream signals to temporally direct the crucial developmental and transitional phases during seed germination and seedling growth, including skotomorphogenesis, photomorphogenesis, and the transition between skotomorphogenesis and photomorphogenesis (Fig. 1).

TOR signaling is essential for seed germination. The downregulation of TOR signaling in the key TOR complex component *raptor1b* mutant triggers high levels of abscisic acid (ABA), auxin, and jasmonic acid (JA) and strongly delays the germination process (Salem *et al.*, 2017). Protein phosphatase 2A (PP2A)-associated protein TIP41 negatively regulates ABA sensitivity, and *tip41* mutants are hypersensitive to the TOR inhibitor AZD8055, delaying seed germination (Punzo *et al.*, 2018). During wheat seed germination, starch stored in the endosperm needs to be broken down into simple sugars to support germination, and the growth hormone gibberellic acid (GA) plays an important role in this process through activating the TF GAMYB to promote α-amylase synthesis (Smailov *et al.*, 2020). Interestingly, TOR has been found to be activated by GA and then to induce the expression of the gene encoding α-amylase to promote germination in wheat seeds (Smailov *et al.*, 2020).

Starting from germination underneath the soil, eudicot plants have to elongate their hypocotyl quickly and efficiently to reach into the light. Ethylene and brassinosteroid (BR) are the key hormones repressing and promoting, respectively, the hypocotyl elongation of etiolated seedlings. Long-term inhibition of TOR might trigger ethylene biosynthesis via the TAP46-ACS2/ACS6 link, which then represses hypocotyl elongation (Zhuo *et al.*, 2020). Glucose is one of the main upstream signals for TOR activation, through glycolysis and the mitochondrial bioenergetics-dependent energy relay (Xiong *et al.*, 2013). A very recent study reveals a non-canonical role of ethylene-insensitive protein 2 (EIN2) as a direct substrate of glucose-activated TOR kinase in controlling hypocotyl elongation, which is independent of ethylene sensing and signaling (Fu *et al.*, 2021). Moreover, sugar–TOR signaling stabilizes the key BR-signaling TF brassinazole resistant 1 (BZR1) by repressing autophagic protein degradation activity, thus

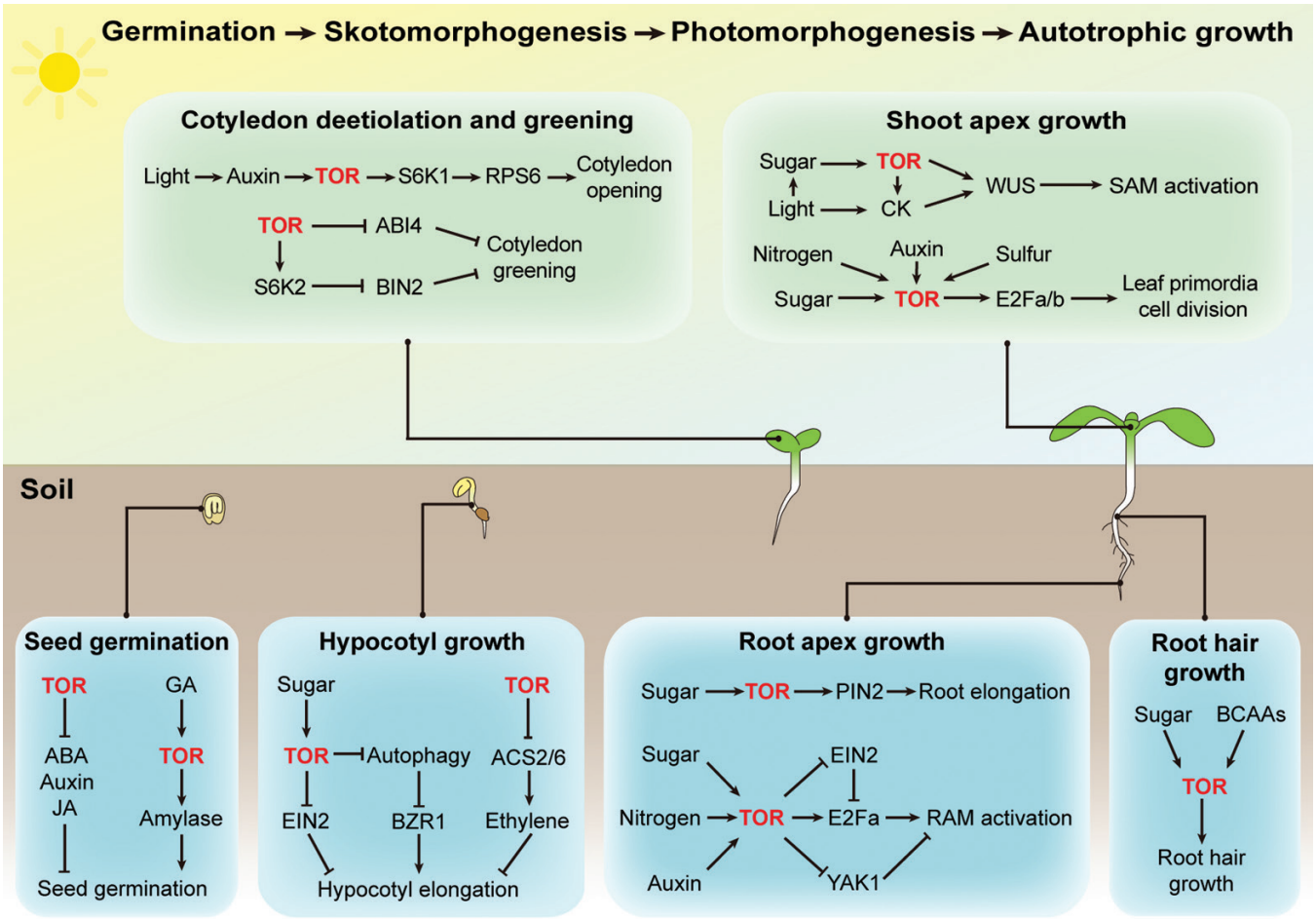

Fig. 1. Target of rapamycin (TOR) signaling in the transition from heterotrophic to autotrophic growth. TOR senses and integrates diverse internal and external upstream signals including sugar, nitrogen, sulfur, amino acids (AAs), auxin, and gibberellic acid (GA) to temporally direct the crucial developmental and transitional phases from, germination, skotomorphogenesis, photomorphogenesis, to autotrophic growth. ABA, abscisic acid; ABI4, ABA-insensitive 4; ACS2/6, ACC synthase 2/6; BCAAs, branched-chain amino acids; BIN2, brassinosteroid-insensitive 2; BZR1, brassinazole resistant 1; CK, cytokinin; E2Fa/b, E2 promoter binding factor a/b; EIN2, ethylene-insensitive protein 2; JA, jasmonic acid; PIN2, PIN-formed 2; RAM, root apical meristem; RPS6, ribosome protein S6; S6K1/2, ribosomal protein S6 kinase 1/2; SAM, shoot apical meristem; WUS, WUSCHEL; YAK1, yet another kinase 1.

activating the expression of BR-responsive genes to promote hypocotyl elongation of etiolated seedlings (Zhang *et al.*, 2016). In contrast to the promotion of hypocotyl elongation in the dark by sugar–TOR signaling, a high level of sugar attenuates the promotion by BR of hypocotyl elongation under light. Intriguingly, this inhibitory effect of sugar is independent of TOR signaling but requires the GSK3-like kinase brassinosteroid-insensitive 2 (BIN2), a negative regulator in the BR pathway (Zhang *et al.*, 2021).

After breaking through the soil, plant seedlings are able to directly sense the light signal to trigger the processes of photomorphogenesis, namely cotyledon opening, chloroplast development, and cotyledon greening (Wu, 2014). The light signal initiates these pivotal developmental processes through massive reprogramming of the transcriptional and translational networks, and TOR is one of the key regulators in these processes. The photoreceptors phytochrome A (phyA) and cryptochromes (CRYs) perceive far-red and blue light, respectively, to inactivate the negative regulator constitutive photomorphogenesis 1 (COP1), which leads to the derepression of the auxin pathway to activate TOR–S6K1-dependent phosphorylation of ribosome protein S6 (RPS6). Phosphorylation of RPS6 enhances translation efficiency to promote cotyledon opening (Chen *et al.*, 2018). In the shoot, chloroplast biogenesis and maturation are essential to establish functional photosynthesis in response to light. TOR increases the expression of genes involved in chloroplast development and photosynthesis. In addition, TOR negatively regulates the TFs ABA-insensitive 4 (ABI4) and BIN2 to regulate chlorophyll biosynthesis and chloroplast formation to promote cotyledon greening (L. Li *et al.*, 2015; Xiong *et al.*, 2017).

During photomorphogenesis, it is important for plants to activate the shoot apex to ensure rapid true leaf growth at this stage, which is controlled strictly by internal developmental cues such as nutrients and hormones, and external environmental signals (Nemhauser and Chory, 2002). TOR plays a pivotal role by integrating different signals to activate the shoot apex (Pfeiffer *et al.*, 2016; Li *et al.*, 2017; Shi *et al.*, 2018). In the shoot apical meristem, the maintenance of stem cells is based on the homeodomain TF WUSCHEL (WUS) (Schoof *et al.*, 2000; Daum *et al.*, 2014). Light–cytokinin signaling and photosynthesis-driven sugar signaling converge on TOR to induce the expression of *WUS* (Pfeiffer *et al.*, 2016). Moreover, in the leaf primordia of shoot apices, both glucose–energy and light–COP1–auxin–ROPs signaling are required for TOR activation. Activated TOR then directly phosphorylates the key TFs E2 promoter binding factor a and b (E2Fa, E2Fb) to promote the expression of cell cycle S-phase genes, thus activating cell proliferation in the leaf primordia for true leaf development (Li *et al.*, 2017; Schepetilnikov *et al.*, 2017). In addition to glucose and light, nitrogen nutrient signals transported from the root have also been found to activate TOR to promote true leaf development. Inorganic nitrogen (nitrate and ammonium) and organic AAs function as separate signals to activate TOR through the GTPase rho-related protein from plants 2 (ROP2) (Liu *et al.*, 2021; Tulin *et al.*, 2021). Sulfur, another plant macronutrient, could also activate TOR and promote plant leaf growth and development via both glucose-energy-dependent and -independent pathways (Dong *et al.*, 2017; Yu *et al.*, 2021, Preprint). Therefore, TOR integrates complex nutrient and hormonal signals to ensure optimal activation of the shoot apex.

The leaf-produced sugars can also be transported to the root and activate TOR for promotion of primary root and root hair growth (Xiong and Sheen, 2012; Xiong *et al.*, 2013). When activated by glucose in the root, TOR phosphorylates the TF E2Fa to support S-phase gene expression, thus promoting root apical meristem (RAM) cell division (Xiong *et al.*, 2013). Meanwhile, TOR also phosphorylates and represses the nuclear localization of EIN2 to enhance *E2Fa* expression to activate the RAM (Fu *et al.*, 2021). In addition, TOR phosphorylates the downstream suppressor yet another kinase 1 (YAK1), which was identified by two independent genetic screening studies. *yak1* mutations confer resistance to the TOR inhibitor AZD8055, suppress *lst8* mutations, and promote cell proliferation in the root meristem by repressing the expression of the CDK inhibitor genes *Siamese-related* (*SMRs*) (Barrada *et al.*, 2019; Forzani *et al.*, 2019). In addition, TOR phosphorylates and stabilizes the auxin efflux carrier PIN-formed 2 (PIN2) to regulate the auxin gradient distribution in the root elongation zone that is essential for cell elongation (Yuan *et al.*, 2020), and senses both sugar and branched-chain AA (BCAA) signals to promote root hair growth and development (Xiong and Sheen, 2012; Cao *et al.*, 2019; Schaufelberger *et al.*, 2019). Together, these results suggest multifaceted functions of TOR that are intertwined with multiple layers of diverse nutrient and hormonal signals to temporally guide plant transition from heterotrophic growth to autotrophic growth during seed germination and seedling growth processes (Fig. 1).

The intimate connection between TOR signaling and the circadian clock

The rotation of the Earth creates daily oscillations of light and temperature. Organisms have evolved an autonomous timekeeping mechanism known as the circadian clock, with a period of ~24 h, to perceive and anticipate the changing environments (Reinke and Asher, 2019; Sanchez *et al.*, 2020). The circadian clock helps organisms to orchestrate rhythmic oscillations of their internal physiological and developmental processes, timing them to the appropriate point of the day (Greenwood and Locke, 2020). This rhythmicity arises from the circadian oscillators, which are mainly encoded by a set of clock genes, forming complex interlocking transcription–translation feedback loops (TTFLs) (Dodd *et al.*, 2015). Recent studies have revealed an intimate relationship between TOR and the circadian clock in both mammals and plants (Cao, 2018; Zhang *et al.*, 2019; Greenwood and Locke, 2020; Eskandari *et al.*, 2021) (Fig. 2).

In mammals, the core circadian oscillators can be simplified into a single mechanism framework: the morning-expressed TFs CLOCK and BMAL1 correlate with the evening-expressed TFs Period (PER) and CRY, to form a reciprocal regulatory loop (Cao, 2018; Reinke and Asher, 2019). Intriguingly, the expression and activity of TOR complex 1 (TORC1; see below for a more detailed description) display a robust circadian rhythm and are strongly relevant to the core oscillator-controlled feeding behaviors. In mice, knockout of *BMAL1* increases TORC1 activity and disrupts its rhythmicity, suggesting that BMAL1 is a negative regulator of TORC1 (Jouffe *et al.*, 2013; Khapre *et al.*, 2014b; Robles *et al.*, 2017). During fasting, PER2 is activated and tethers tuberous sclerosis complex 1 (TSC1) to TORC1, thus inhibiting TORC1 activity (R. Wu *et al.*, 2019). Furthermore, TORC1 exhibits lower activity during the day and higher activity during the night, and this temporal oscillation of TORC1 activity is well correlated with the rhythmic oscillation of TORC1-regulated downstream biological activities, such as ribosome biogenesis, protein translation, and autophagy (Jouffe *et al.*, 2013; Khapre *et al.*, 2014a; Robles *et al.*, 2017; R. Wu *et al.*, 2019). TORC2 activity and expression also display diurnal oscillation, although the detailed mechanisms are still unclear (Dragert *et al.*, 2016). It will be worth investigating whether TORC2-regulated downstream processes also have a circadian rhythm. Moreover, as well as serving as a downstream output of the circadian oscillator, TOR can also set the pace of the clock. Treatment with TOR inhibitors (e.g. rapamycin or Torin1) significantly lengthens the circadian period (Feeney *et al.*, 2016; Ramanathan *et al.*, 2018).

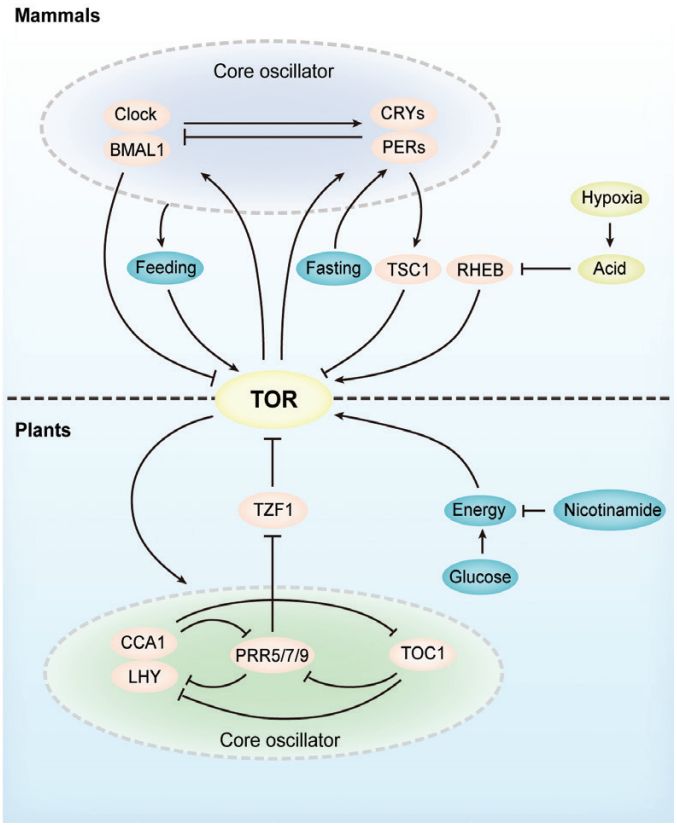

Fig. 2. Reciprocal regulation between TOR signaling and the circadian clock. There is an intimate relationship between metabolism, TOR, and the circadian clock in both mammals and plants. In mammals, the expression and activity of TOR display a robust circadian rhythm and are strongly relevant to the core oscillator-controlled feeding and fasting behaviors. In turn, TOR can also set the pace of the clock by regulating the expression levels of core oscillators. In plants, TOR senses glucose- and nicotinamide-influenced energy signals to orchestrate the circadian period, while the core circadian oscillators PRR5/7/9 promote *TOR* mRNA stability through directly inhibiting the expression of the negative regulator *TZF1*. BMAL1, brain and muscle ARNT-like 1; CCA1, circadian clock associated 1; Clock, Circadian locomotor output cycles kaput; CRYs, cryptochromes; LHY, late elongated hypocotyl; PERs, Periods; PRR5/7/9, Pseudo-response regulator 5/7/9; RHEB, Ras homolog enriched in brain; TOC1, timing of CAB expression 1; TSC1, tuberous sclerosis complex 1; TZF1, tandem zinc finger 1.

Hypoxia-induced acidulation could repress TORC1 through its disassociation from the activator RHEB and then trigger translational inhibition of the core clock genes *BMAL1*, *CRY2*, and *PER2* (Walton *et al.*, 2018), while constitutive activation of TORC1 elevates the expression levels of *CRY1*, *BMAL1*, and *CLOCK* (Ramanathan *et al.*, 2018) (Fig. 2). Further studies have also shown that attenuated expression of *TOR* lengthens the circadian period of locomotor activity in mice, supporting a significant role for TOR in circadian timekeeping across physiology to behavior (Ramanathan *et al.*, 2018).

Plants have evolved unique clock genes to guide the daily oscillations of metabolism and physiology, as most of the mammalian clock genes have no plant homologs (Greenham and McClung, 2015; Creux and Harmer, 2019). In brief, the dawn-expressed MYB family TFs circadian clock associated 1 (CCA1) and late elongated hypocotyl (LHY) repress the morning- to dusk-expressed pseudo-response regulator (PRR) family TFs. In turn, PRR5/7/9 and timing of CAB expression 1 (TOC1; also known as PRR1) inhibit the transcription of *CCA1* and *LHY*, thereby setting up the plant TTFLs (Greenwood and Locke, 2020; Sanchez *et al.*, 2020). Increasing evidence has shown that metabolic processes are not simply downstream outputs of the circadian clock, as some key metabolites, such as photosynthesis-produced sugars, serve as core inputs for clock pace setting (Haydon et al., 2013a, b). Interestingly, inhibition of TOR severely blocks the glucose-regulated circadian period in Arabidopsis and significantly delays the period length, suggesting that TOR is also a key clock regulator in plants (Zhang *et al.*, 2019; Wang *et al.*, 2020). Further study revealed that regulation of the circadian clock by TOR relies on the glucose-energy relay (Zhang *et al.*, 2019). Another metabolite, nicotinamide (vitamin B3), a key precursor of nicotinamide adenine dinucleotide (NAD^+), blocks glucose promotion of energy production, TOR activation, and glucose–TOR-regulated circadian period and root meristem activity (Stein and Imai, 2012; Zhang *et al.*, 2019), indicating a pivotal role of TOR in integrating dynamic metabolic status to coordinate the circadian clock and appropriate plant growth during a 24 h cycle. Besides, the core TTFL components PRR5/7/9 directly repress tandem zinc finger 1 (TZF1), a negative regulator of *TOR* mRNA stability, to promote *TOR* expression and activation of the root meristem (Li *et al.*, 2019) (Fig. 2). Another study revealed that TOR-dependent phosphorylation of RPS6 seems to show a diel oscillation (Enganti *et al.*, 2017). Future study would be required to fully understand the connection and regulatory mechanism between TOR and the circadian clock.

TOR spatially regulates tissue-specific responses

Different parts and stages of a plant may trigger distinct molecular responses and developmental programs in response to varying environmental conditions. The shoot and root apices share highly similar molecular players encoded by closely related and tissue-specific homologs, but are spatially separated by soil. Interestingly, these two meristematic tissues have differential activation requirements for sugar and light signals (Pfeiffer *et al.*, 2016; Li *et al.*, 2017). In harmony with nature, the shoot apex demands light for the activation of cell proliferation during true leaf organogenesis. By contrast, glucose alone could activate cell proliferation in the root apex and promote rapid primary root growth, even in the absence of light (Li *et al.*, 2017). Moreover, glucose alone can efficiently activate TOR kinase in the root apex, while neither light nor

glucose alone can activate TOR kinase in the leaf primordium of the shoot apex. Further investigation revealed that both glucose and auxin are essential upstream signals for TOR kinase activation. In the root meristem, a relatively high local auxin synthesis and sensitivity to auxin with the presence of glucose is sufficient to activate TOR kinase in the dark (or under the soil) (Stepanova *et al.*, 2008). However, in the aerial shoot apex, light is indispensable for continuously promoting auxin synthesis and accumulation for TOR activation (Li *et al.*, 2017) (Fig. 3A). Intriguingly, several studies also reported that sugar alone can promote some meristematic activity and shoot organ development in the dark, which might be caused by different seedling stages, sugar deprivation, and dark treatment conditions (Roldán *et al.*, 1999; Mohammed *et al.*, 2018). It would be interesting to examine whether sufficient auxin accumulates in the leaf primordia region to overcome dark morphogenetic arrest by the presence of exogenous sucrose with delayed germination and seedling development in darkness. These various outcomes of light, hormones, and energy signaling for shoot organ initiation and development suggest that multifaceted and complex regulatory mechanisms for this key developmental process respond to dynamic environmental changes (Roldán *et al.*, 1999; Pfeiffer *et al.*, 2016; Li *et al.*, 2017; Mohammed *et al.*, 2018).

Another well-documented example of tissue-differential responses is that nitrogen limitation significantly decreases the shoot-to-root ratio, caused by different root and shoot growth patterns: the primary root grows rapidly but shoot growth is strongly impaired (Ruffel *et al.*, 2011; Liu *et al.*, 2021). Consistent with these different growth patterns, Liu *et al.* (2021) recently reported that the cell proliferation and TOR kinase activity are abolished only in the shoot apex but not in the root apex under conditions of limited inorganic nitrogen supply. They further found that in addition to glucose and auxin, nitrogen nutrition (including nitrate, ammonium, and multiple AAs) is another major indispensable upstream signal for TOR kinase activation. Metabolomic analyses further discovered that under limited inorganic nitrogen supply conditions, although the nitrate level is decreased to an undetectable level in both the root and the shoot apex, ammonium and the AAs with high potency for TOR activation are still maintained at high levels in roots, compared with their levels in shoots. These high levels of ammonium and AAs in the root apex, therefore, could maintain high TOR activity in the root apex to promote cell proliferation and support rapid root growth (Liu *et al.*, 2021) (Fig. 3B).

Different parts and stages of a plant also need to communicate and exchange cargos, for example, carbohydrates, proteins, and hormones, which is done mainly through the specialized vascular tissue, phloem. Via this long-distance transport system, plants can redistribute photosynthesized sugar from mature leaves to young leaves and roots to support their development, a process called source-to-sink redistribution (Brunkard, 2020). In mature source leaves, excess sugars produced by photosynthesis are loaded into the phloem by active transporters. Moreover, to prevent passive backflow, the activity of the plasmodesmata (PD), nanoscopic membrane-bound channels, is tightly restricted. In young sink leaves, increased PD transport can facilitate rapid and passive relocation of highly concentrated sugars out from the phloem into neighboring young leaf cells (Brunkard *et al.*, 2020). A recent elegant study revealed that TOR kinase activity negatively correlates with PD transport through an as yet unknown mechanism. Interestingly, TOR kinase activity positively correlates with leaf age, possibly because the young leaves consume sugar rapidly, thus TOR is relatively inactive, whereas in mature leaves, sugar is synthesized in excess, thus TOR is strongly activated (Brunkard *et al.*, 2020). This dynamic change of TOR activity between mature and young leaves allows TOR to act as a rheostat to regulate the PD transport activity, and therefore coordinate the transition from young sink to mature source leaves during plant vegetative development (Fig. 3C).

Although they lack the neuron system that exists in mammals, plants seem to have developed a subtle mechanism to modulate TOR kinase activity based on the unequal local sensitivity to or distribution of auxin, nitrogen, and sugar in response to diverse environmental conditions and developmental stages, which allows plant cells to 'recognize' their spatial location. It will be interesting to investigate whether other signaling molecules, such as the hormones cytokinin and BR or even some mobile TFs, also have an unequal local distribution in different organs/tissues, thereby mediating TOR signaling to modulate plant growth and development in response to the dynamic environment.

How does TOR recognize and distinguish diverse upstream signals?

TOR is able to recognize and distinguish diverse upstream signals by forming distinct TOR protein complexes. In mammals, two structurally and functionally distinct protein complexes have been well characterized, termed mammalian/mechanistic TOR complex 1 and 2 (mTORC1 and mTORC2) (Mossmann *et al.*, 2018; Liu and Sabatini, 2020). The core components of mTORC1 are mTOR, lethal with SEC13 protein 8 (LST8), and regulatory-associated protein of mTOR (RAPTOR). mTORC2 , like mTORC1, contains TOR and LST8, while rapamycin-insensitive companion of mTOR (RICTOR) and SAPK-interacting protein 1 (SIN1) are unique to mTORC2 (Liu and Sabatini, 2020). The TORC1 subunits LST8 and RAPTOR are present in all sequenced plant species, whereas RICTOR and SIN1 appear to be absent, indicating that TORC2 might not be conserved in plants (Y. Wu *et al.*, 2019; Burkart and Brandizzi, 2021). Intriguingly, unlike the embryonic lethality of the *tor* null mutant, Arabidopsis *raptor* mutants exhibit normal embryonic development (Anderson *et al.*, 2005), suggesting that in plants, TOR's function is not

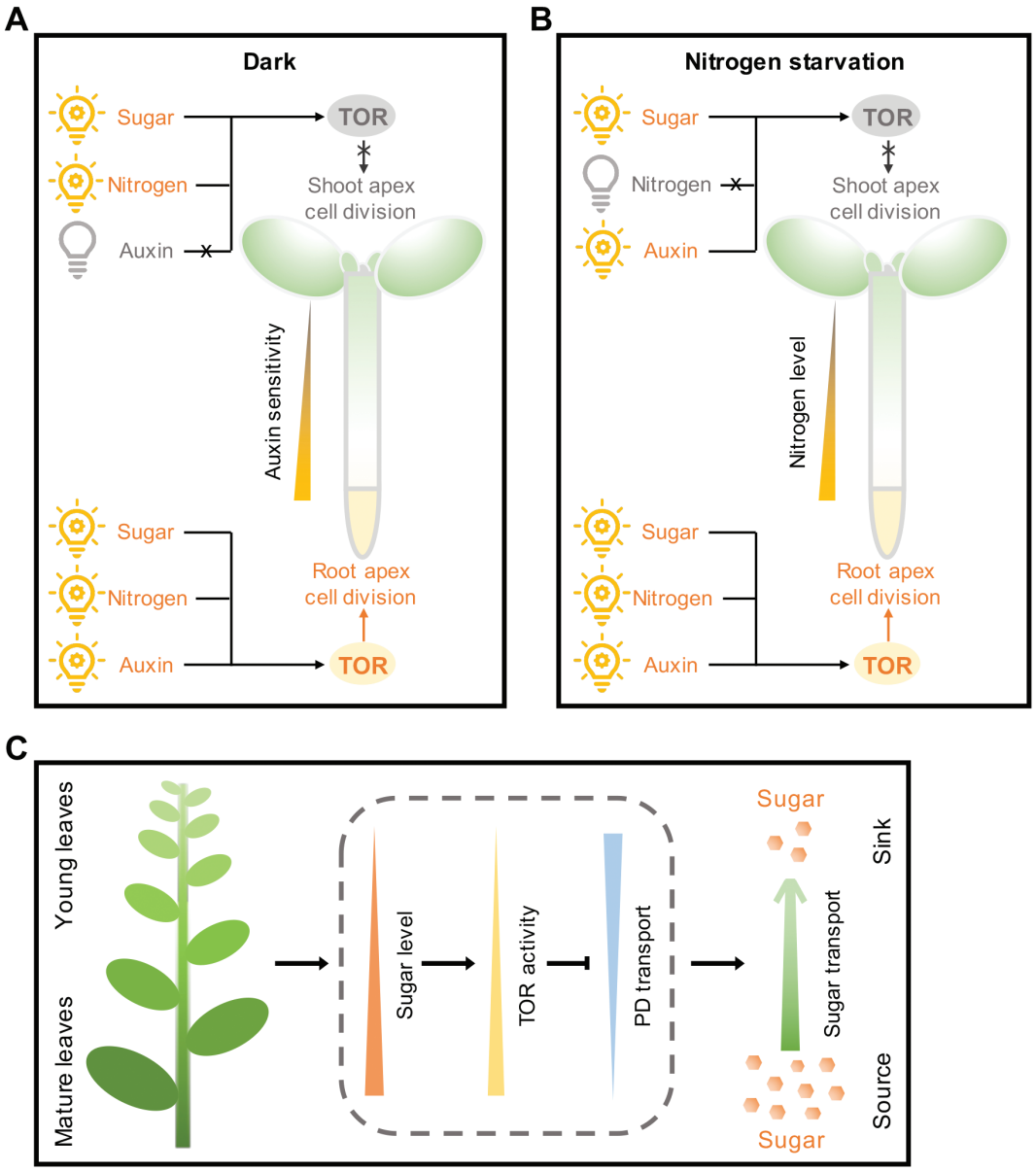

Fig. 3. TOR spatially regulates tissue-specific responses. Sugar, nitrogen, and auxin are all essential upstream signals for TOR kinase activation. Plants have developed a subtle mechanism to 'recognize' their spatial location based on the unequal local sensitivity to or distribution of sugar, nitrogen, and auxin, and therefore specifically activate cell proliferation only in the root apex but not in the shoot apex in the dark (A) and under limited inorganic nitrogen supply conditions (B), or differentially regulate the plasmodesmata (PD) transport activity in young and mature leaves to coordinate the redistribution of sugar from mature source leaves to young sink leaves (C). In (A) and (B), orange represents the sufficient or activated state, and grey represents the limited or inactivated state; for (C), hexagons represent sugar molecules.

fully dependent on RAPTOR, and there may exist functionally equivalent TORC2 or even novel TOR complexes with plant specific partners.

Different TOR complexes with unique components provide one of the molecular bases for recognizing different upstream inputs. In mammals, AAs are sensed by different sensors and then converge on the activation of heterodimeric RAG GTPases in a RAPTOR-dependent manner (Zoncu *et al.*, 2011; Shi *et al.*, 2018). TORC2 has not been reported to response to AAs. By contrast, insulin or serum is sufficient to activate TORC2 through the production by phosphoinositide 3-kinase (PI3K) of phosphatidylinositol-(3,4,5)-triphosphate (PIP3), which in turn binds SIN1 to relieve a SIN1-mediated inhibitory effect on TORC2 (Zinzalla *et al.*, 2011; Yuan and Guan, 2015; Mossmann *et al.*, 2018; Liu and Sabatini, 2020). In plants, TORC1 has been reported to receive different upstream signals via different TORC1 components (Fu *et al.*, 2020). The energy sensor Snf1-related kinase 1 (SnRK1), a homolog of

AMP-activated kinase (AMPK) in mammals, is a conserved TORC1 repressor in almost all eukaryotes (Jamsheer *et al.*, 2019; Gonzalez *et al.*, 2020). Under energy-deprived conditions, Arabidopsis SnRK1 is activated and directly phosphorylates RAPTOR to inhibit TORC1 activity (Nukarinen *et al.*, 2016). Similarly, SnRK2s activated by the plant stress hormone ABA are also able to directly phosphorylate RAPTOR or release the activated SnRK1 to phosphorylate RAPTOR, thereby repressing TOR signaling (Wang *et al.*, 2018; Belda-Palazon *et al.*, 2020). Moreover, in *Chlamydomonas*, phosphate starvation triggers a sharp decrease in LST8 abundance, thus dampening TORC1 activity, which is mediated by a key TF, phosphate starvation regulator 1 (PSR1) (Couso *et al.*, 2020).

Changes in subcellular localization provide another means to recognize and respond to different upstream signals (Pacheco *et al.*, 2021). In mammals, AAs and growth factors such as insulin can anchor inactive cytosolic mTORC1 to the lysosomal surface for its final activation, whereas insulin or serum promotes the plasma membrane or ribosome localization of mTORC2 (Zinzalla *et al.*, 2011; Yuan and Guan, 2015; Mossmann *et al.*, 2018; Liu and Sabatini, 2020). In yeast, active TORC1s have been found to be located on the vacuole and endosomes, where they stimulate protein-synthesis-related anabolism and repress autophagy-related catabolic processes, respectively (Hatakeyama and De Virgilio, 2019). Furthermore, TOR protein has also been found to be dynamically distributed in the cytoplasm and the nucleus. Nutrient repletion can trigger yeast Tor1 nuclear localization, and nuclear Tor1 can bind to 35S ribosomal DNA (rDNA) to promote 35S rRNA synthesis and cell growth (Li *et al.*, 2006). In plants, auxin is reported to activate ROP2, which then binds and targets TOR to the endosome-like structures in Arabidopsis (Schepetilnikov *et al.*, 2017). Arabidopsis TOR-GFP fusion protein has also been observed in both the cytosol and the nucleus when transiently expressed in onion epidermal cells. This nuclear-localized TOR directly binds to the 45S *rRNA* promoter to regulate rRNA synthesis (Ren *et al.*, 2011), suggesting a conserved nuclear function of TOR between plants and yeasts. In addition, Arabidopsis TOR and RAPTOR1B have also been identified in the Golgi and plastids by proteomic studies (Tomizioli *et al.*, 2014; Heard *et al.*, 2015), although the related biological functions have not been explored yet.

How does TOR control different downstream functions?

Recent studies have started to reveal how TOR kinase could act as a master regulator to differentially control a broad spectrum of substrates and effectors in response to diverse positive and negative inputs. First of all, different TOR complexes are able to recognize and regulate different downstream substrates. In mammals, mTORC1 promotes protein translation and ribosomal biogenesis by phosphorylating eIF4E-binding proteins (4EBP1) and ribosomal protein S6 kinase 1 (p70 S6K1) (Sarbassov *et al.*, 2004; Gonzalez *et al.*, 2020; Liu and Sabatini, 2020). Furthermore, mTORC1 drives lipid and nucleotide biosynthesis by activating the TFs sterol regulatory element binding protein (SREBP) and ATF4, respectively (Peterson *et al.*, 2011; Ben-Sahra *et al.*, 2016), and inhibits autophagy by phosphorylating the unc-51-like autophagy-activating kinase 1 (ULK1) and ATG13 (Kim *et al.*, 2011). Meanwhile, mTORC2 regulates cytoskeleton remodeling via protein kinase C (PKC) (Sarbassov *et al.*, 2004), and mutation of RICTOR but not RAPTOR impairs this reorganization of the cytoskeleton network (Jacinto *et al.*, 2004). In Arabidopsis, TOR phosphorylates S6K1 in a RAPTOR-dependent manner, indicating a conserved downstream function of TORC1 (Mahfouz *et al.*, 2006). The function of TOR in regulating the actin cytoskeleton seems to be also conserved in plants. BCAAs could control TOR signaling to induce actin bundling in Arabidopsis cotyledon epidermal cells. Interestingly, this BCAA regulation of the temporal reorganization of the cytoskeleton requires TOR but is not disrupted in the *raptor1b* mutant (Cao *et al.*, 2019), further supporting the possibility that plants may harbor TOR complexes functionally equivalent to TORC2 in yeasts or mammals.

Diverse tissue and subcellular expression patterns could also contribute to substrate specificities. Plant TOR substrates and effectors have been identified or predicted to be localized in various subcellular locations; for example, E2Fa and E2Fb in the nucleus, EIN2 in the endoplasmic reticulum (ER) membrane, and PIN2 in the plasma membrane (Xiong *et al.*, 2013; Yuan *et al.*, 2020; Fu *et al.*, 2021). Furthermore, these TOR targets might also have tissue- and organ-specific expression patterns; for example, PIN2 is reported to be highly accumulated in the root (Luschnig *et al.*, 1998; Yuan *et al.*, 2020). In Table 1, we summarize the tissue/organ expression patterns of known direct targets of TOR based on the developmental map in the Arabidopsis eFP Browser (http://bar.utoronto.ca/) (Winter *et al.*, 2007). Interestingly, TOR has higher activity in the mature leaf than in the young leaf (Brunkard *et al.*, 2020). However, the activities of S6K1 exhibit only a subtle difference, which may be caused by the significant accumulation of S6K1 protein in young leaves (Brunkard, 2020; Brunkard *et al.*, 2020), indicating a compensation mechanism that can maintain homeostatic balance when TOR activity is reduced.

'Substrate quality' provides another mechanism for allowing TOR downstream effectors to respond differentially to diverse positive and negative inputs. Rapamycin inhibits mTORC1 function only partially. Intriguingly, several known mTORC1 substrates have been found to be either rapamycin-sensitive or -insensitive. For example, mTORC1-dependent p70 S6K1-T389 phosphorylation is almost completely blocked by rapamycin treatment, whereas ULK1-S758 phosphorylation shows prominent resistance to rapamycin in mammals (Kang *et al.*, 2013). Recent studies have decoded the inherent capacity of various

Table 1. The tissue expression patterns of identified TOR substrates.

Protein	Locus	Brief description	Expression					References
					Min → Max			
			Seed	Root	Stem	Leaf	Flower	
TAP46	AT5G53000	PP2A-associated protein with a possible function in the chilling response						(Ahn *et al.*, 2011)
S6K1	AT3G08730	Encodes a protein -serine kinase that phosphorylates ribosomal protein *in vitro*						(Mahfouz *et al.*, 2006)
S6K2	AT3G08720	Encodes a ribosomal -protein S6 kinase. Gene expression is induced by cold and salt (NaCl)						(Xiong *et al.*, 2017)
E2Fa	AT2G36010	Cell cycle genes, key components of the cyclin D/retinoblastoma/E2F pathway						(Xiong *et al.*, 2013)
E2Fb	AT5G22220	Key components of the cyclin D/retinoblastoma/E2F pathway. Binds DPA and RBR1 proteins						(Li *et al.*, 2017)
ATG1B	AT3G53930	Protein kinase superfamily protein						(Van Leene *et al.*, 2019)
ATG13	AT3G49590	Autophagy protein						(Van Leene *et al.*, 2019)
EIN2	AT5G03280	Involved in ethylene signal transduction. Acts downstream of CTR1						(Fu *et al.*, 2021)
YAK1	AT5G35980	YAK1 mutations suppress TOR deficiency in Arabidopsis and consequences of *lst8* mutations						(Forzani *et al.*, 2019)
PIN2	AT5G57090	Encodes an auxin efflux carrier that is similar to bacterial membrane transporters						(Yuan *et al.*, 2020)
PYL1	AT5G46790	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL2	AT2G26040	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL3	AT1G73000	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL4	AT2G38310	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL5	AT5G05440	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL6	AT2G40330	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL7	AT4G01026	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL8	AT5G53160	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYL9	AT1G01360	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PYR1	AT4G17870	PYR/PYL/RCAR family proteins function as abscisic acid sensors						(Wang *et al.*, 2018)
PH-DUF828	AT5G43870	FORKED-LIKE family member, may coordinate leaf size with vein density						(Van Leene *et al.*, 2019)
S40-7	AT3G15040	Senescence regulator						(Van Leene *et al.*, 2019)
LARP1a	AT5G21160	Involved in mRNA degradation in response to heat stress						(Scarpin *et al.*, 2020)
AT3G50370	AT3G50370	Hypothetical protein						(Van Leene *et al.*, 2019)
eIF2B-δ1	AT5G38640	NagB/RpiA/CoA transferase-like superfamily protein						(Van Leene *et al.*, 2019)
AML5	AT1G29400	A member of the *mei2-like* gene family, encoding RNA -binding proteins						(Van Leene *et al.*, 2019)

The tissue expression patterns of these TOR substrates are were obtained from the Ddevelopmental Mmap in the Arabidopsis eFP Browser (http://bar.utoronto.ca/). Each protein's tissue expression level is relative to itself,, with the color gradient from yellow to red representsing the expression level from minimum (min) to maximum (max). These direct TOR substrates are identified according to the references in theis table.

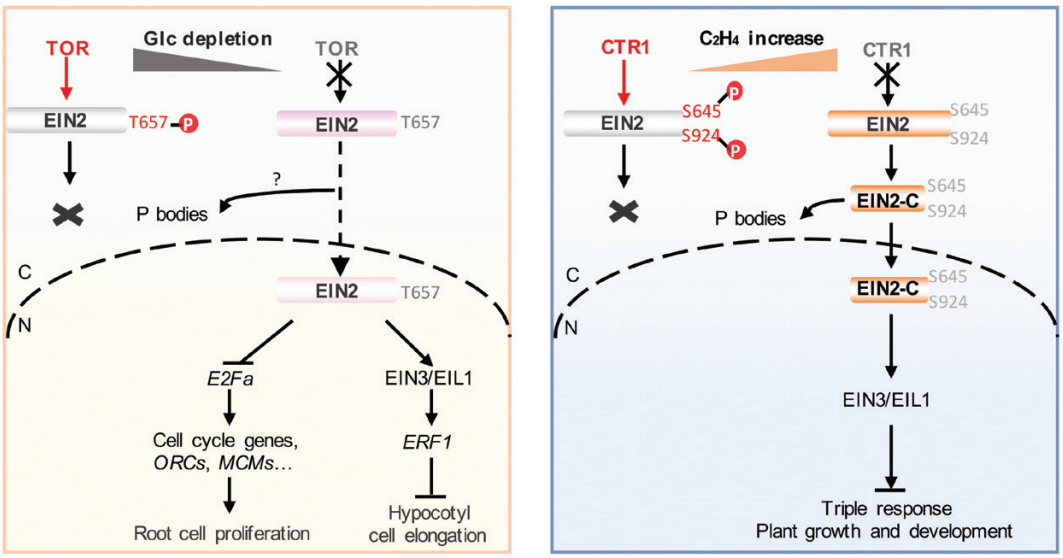

Fig. 4. Glucose–TOR signaling interconnects with the ethylene signaling pathway via a 'phosphorylation code' mode. Glucose (Glc), via TOR kinase regulation, and ethylene (C_2H_4), via CTR1 kinase regulation, control distinct phosphorylation sites in EIN2. Ethylene-induced dephosphorylation of EIN2 triggers proteolysis of EIN2, and the C-terminus of EIN2 moves into the nucleus. By contrast, inhibition of TOR rapidly triggers the accumulation of full-length EIN2 in the nucleus, without cleavage. These distinct dephosphorylated forms of EIN2 could control overlapping and different gene expression programs to orchestrate complex plant growth and development. C, cytoplasm; CTR1, constitutive triple response 1; E2Fa, E2 promoter binding factor a; EIN2, ethylene-insensitive protein 2; EIN3/EIL1, ethylene insensitive 3 and ethylene insensitive 3-like 1; ERF1, ethylene response factor 1; MCM, minichromosome maintenance; N, nucleus; ORC, origin recognition complex.

sequence compositions in protein motifs of mTORC1 substrates to serve as a quality control (good or poor substrate) for determining the substrate affinity with mTORC1, and thereby the sensitivity to rapamycin as well to as nutrients and growth factors (Kang *et al*., 2013). mTORC1 is sufficient to keep good substrates phosphorylated, even with reduced kinase activity, for example, when treated with rapamycin or under conditions of mild nutrient deprivation. By contrast, poor substrates with weak affinity with mTORC1 would require higher or even fully activated mTORC1 to keep them phosphorylated, and therefore they exhibit acute rapamycin sensitivity. Interestingly, more complex mTOR substrates, such as 4EBP1, LIPIN1, and GRB10, possess both low-affinity rapamycin-sensitive and high-affinity rapamycin-resistant phosphorylation motifs (Kang *et al*., 2013), supporting that such 'substrate quality' is encoded at the level of an individual phosphorylation site rather than a full-length protein. Therefore, these complex substrates render graded or distinct regulation dependent on different levels of TOR kinase activities to adjust their functional requirement from cell proliferation, growth, and maintenance to the extreme state in stress adaptation. In Arabidopsis, the identification of TOR-regulated proteins by a quantitative phosphoproteomic study also revealed several rapamycin-sensitive or rapamycin-insensitive TOR substrates (Van Leene *et al*., 2019), indicating that there are differences in quality and affinity among plant TOR substrates, which need future functional investigation.

Phosphorylation code to precisely distinguish multiple upstream signals

Current research emphasizes signaling cross-talk, but how the key signaling hubs can discern and respond to multiple upstream signals differentially is still largely unclear. A very recent study revealed that the glucose–TOR signaling pathway directly communicates with the central ethylene signaling protein EIN2 to control hypocotyl elongation and root meristem activation (Fu *et al*., 2021). Strikingly, this glucose–TOR–EIN2 axis controlling cell elongation and proliferation is decoupled from the conventional role of EIN2 in ethylene signaling via distinct phosphorylation sites and downstream molecular effectors. EIN2 links ethylene sensing in the ER to transcriptional regulation in the nucleus through a 'cleave and shuttle' mechanism (Ju *et al*., 2012; Qiao *et al*., 2012). Without ethylene, CTR1 kinase phosphorylates EIN2 at S645 and S924 to retain its subcellular localization on the ER. Upon ethylene perception, the inactivation of CTR1 abolishes the phosphorylated state of EIN2, leading to its cleavage from the ER and followed by shuttling into the nucleus. Interestingly, glucose–TOR regulates EIN2 in a similar nucleus shuttling pattern but without the cleavage, and the TOR phosphorylation site on EIN2 is different from those of CTR1 on EIN2 (Fig. 4). Under glucose-rich conditions, TOR phosphorylates EIN2 on T657 to retain its cytosolic location, whereas the inactivation of TOR by glucose depletion or rapamycin treatment quickly triggers the accumulation of full-length EIN2 in the nucleus.

Glucose–TOR signaling regulates some shared molecular and physiological events with ethylene signaling such as EIN3 protein accumulation, *EBF1* gene expression, and hypocotyl elongation in etiolated seedlings via the EIN2–EIN3 axis (Fig. 4). However, glucose–TOR and ethylene also regulate different molecular functions and physiological events via the distinctly dephosphorylated EIN2. Inhibition of cell proliferation in the root meristem by glucose depletion requires only EIN2 but not EIN3/EIL, and is independent of the ethylene pathway (Fig. 4). Transcriptome comparison further revealed that less than one-third of the transcriptome overlaps between glucose–TOR signaling and ethylene treatment, further supporting that distinct dephosphorylated forms of EIN2 are specialized in performing different functions. Furthermore, manipulation of glucose–TOR-controlled phosphorylation sites in EIN2 successfully uncouples cell growth and proliferation regulated by glucose–TOR and ethylene (Fu *et al.*, 2021). These results provide a mechanistic model for how a central signaling hub is shared but differentially modulated by nutrient and hormonal signaling pathways using distinct 'phosphorylation codes' that can be decoded by specific upstream kinases (Fig. 4).

Conclusions and perspectives

TOR kinase is a highly connected signaling hub coordinating a myriad of internal and external signals to promote growth and sustain life in all eukaryotes. In contrast to the conventional view that its kinase activity is generally in the 'off' or 'on' state, recent studies have provided compelling evidence that the activities of TOR kinase can be adjusted dynamically in different levels in response to specific nutrient and growth stimuli. Different TOR complexes and subcellular locations further represent different physiological states associated with distinct cellular functions, and are regulated in response to a plethora of positive and negative input signals to execute appropriate and distinct downstream biological processes. Functionally equivalent TORC2 and even specific TOR complexes with different partners are likely to exist in plants. Such distinct elements in plant TOR complexes may enable plants to sense and manipulate the unique challenges and stimuli that result from their sessile lifestyle. It will also be of great interest to experimentally investigate and verify the detailed tissue and subcellular expression pattern of TORC components, and the upstream regulators and downstream effectors in response to different inputs, to fully understand the complicated regulatory functions of TOR. We have discussed how TOR could act like a GPS to spatiotemporally integrate the unequal local distribution of upstream signals to precisely direct the appropriate plant developmental and physiological responses at the tissue and organ levels. In the future, the development of new and sensitive technologies for single-cell-based TOR activity observation and functional perturbation of TOR will enable us to dissect the GPS-like function of TOR among diverse cell types in the same tissue, such as proliferating cells in the root meristem zone, elongating cells in the elongation zone, and mature cells in the differentiation zone.

There is an intimate relationship between metabolism, TOR expression and activity, and the circadian clock in plants and mammals. Recent studies have revealed the phenomenon that the circadian rhythm also exists in non-nuclear cells, which cannot perform transcription (O'Neill and Reddy, 2011), implying that there is an independent 'metabolic oscillator' operating in parallel with the canonical TTFL-driven clock system. TOR may serve as a nexus to bridge the TTFL-driven and metabolism-associated clocks. TOR and the circadian clock are both central regulators for sensing internal and external cues to guide plant growth and development; future studies of the interplay between TOR and the circadian clock would provide new insights and strategies in modern agriculture for *de novo* domestication.

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Author contributions

YM, NZ, JS, and YX conceived the review topic and structure; YM, NZ, JS, and YX wrote the review; XS, JL, NZ, and YM prepared the figures and table. All the authors have read and approved the review.

Conflict of interest

The authors declare no conflicts of interest.

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