Trends in Biochemical Sciences

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Review

A Tour of TOR Complex Signaling in Plants

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To identify the appropriate times for growth and development, organisms must sense and process information about the availability of nutrients, energy status, and environmental cues. For sessile eukaryotes such as plants, integrating such information can be critical in life or death decisions. For nearly 30 years, the conserved phosphatidylinositol 3-kinase-related protein kinases (PIKKs) target of rapamycin (TOR) has been established as a central hub for integrating external and internal metabolic cues. Despite the functional conservation across eukaryotes, the TOR complex has evolved specific functional and mechanistic features in plants. Here, we present recent findings on the plant TOR complex that highlight the conserved and unique nature of this critical growth regulator and its role in multiple aspects of plant life.

Highlights

Plant and mammalian target of rapamycin (TOR) signaling pathways share many conserved methods of activation, inhibition, and effectors, and regulate some overlapping cellular processes; however, some of the mammalian TOR core components are missing in plants.

TOR signaling in plants has evolved to regulate multiple plant-specific processes, some using known TOR effectors from mammalian TOR signaling.

Crosstalk between TOR and phytohormone signaling enables plants to modulate the growth response to adapt to a variety of biotic and abiotic stressors.

Should I Stay or Should I Grow Now?

All living organisms need to be able to identify when nutrient availability and environmental cues are conducive to growth and development. In eukaryotes, the conserved target of rapamycin (TOR) kinase serves as a key regulator of growth by receiving inputs from numerous metabolic processes and environmental cues. When conditions are favorable for growth, TOR is active and promotes anabolic processes to drive growth while repressing catabolic processes, but when nutrients are limited or environmental stresses are present, TOR is inactivated and catabolic processes are promoted [1,2].

The yeast *Saccharomyces cerevisiae* has two TOR genes: *TOR1* and *TOR2*. Either TOR1 or TOR2 can form the rapamycin-sensitive TOR complex 1 (TORC1), while only TOR2 can form the rapamycin-insensitive TORC2 [3]. In mammalian systems, there is only one *TOR* gene, but the protein exists in two complexes that are similar to those in yeast, TORC1 and TORC2 [4]. These two signaling complexes differ in core components and the substrates and cellular processes that they affect. Aside from TOR, the core components of both mammalian and yeast TORC1 include **regulatory-associated protein of mTOR (RAPTOR)**/KOG1 (see Glossary) and **lethal with SEC13 protein 8 (LST8)**, while TORC2 includes **SAPK-interacting protein 1 (SIN1)/**AVO1, **rapamycin-insensitive companion of mTOR (RICTOR)**/AVO3, and LST8 [5] (Figure 1). While all plant systems examined to date have homologs of the TORC1 components RAPTOR and LST8, SIN1 and RICTOR appear to be absent in the plant lineage (Figure 2), indicating that plants may not form a conserved TORC2 [6].

TORC1 is the better understood TOR signaling complex and is involved in the regulation of a host of cellular and developmental processes conserved between animals and plants, such as growth and proliferation, protein translation, cell cycle, embryogenesis, stress responses, regulation of stem cells, and biosynthesis of nucleotides, lipids, and amino acids (Figure 1A) [1,7,8]. Metazoan-specific processes controlled by TORC1 include lysosome biogenesis, neural development, and oncogenesis, while plant-specific examples include cell wall assembly, activation of meristems, growth of roots and leaves, and flowering [9,10]. TORC2 in mammalian cells regulates processes such as cell survival, cell migration, focal adhesions, and metabolism of glucose and lipids (Figure 1B) [11,12]. In yeast, TORC2 regulates processes such as turgor

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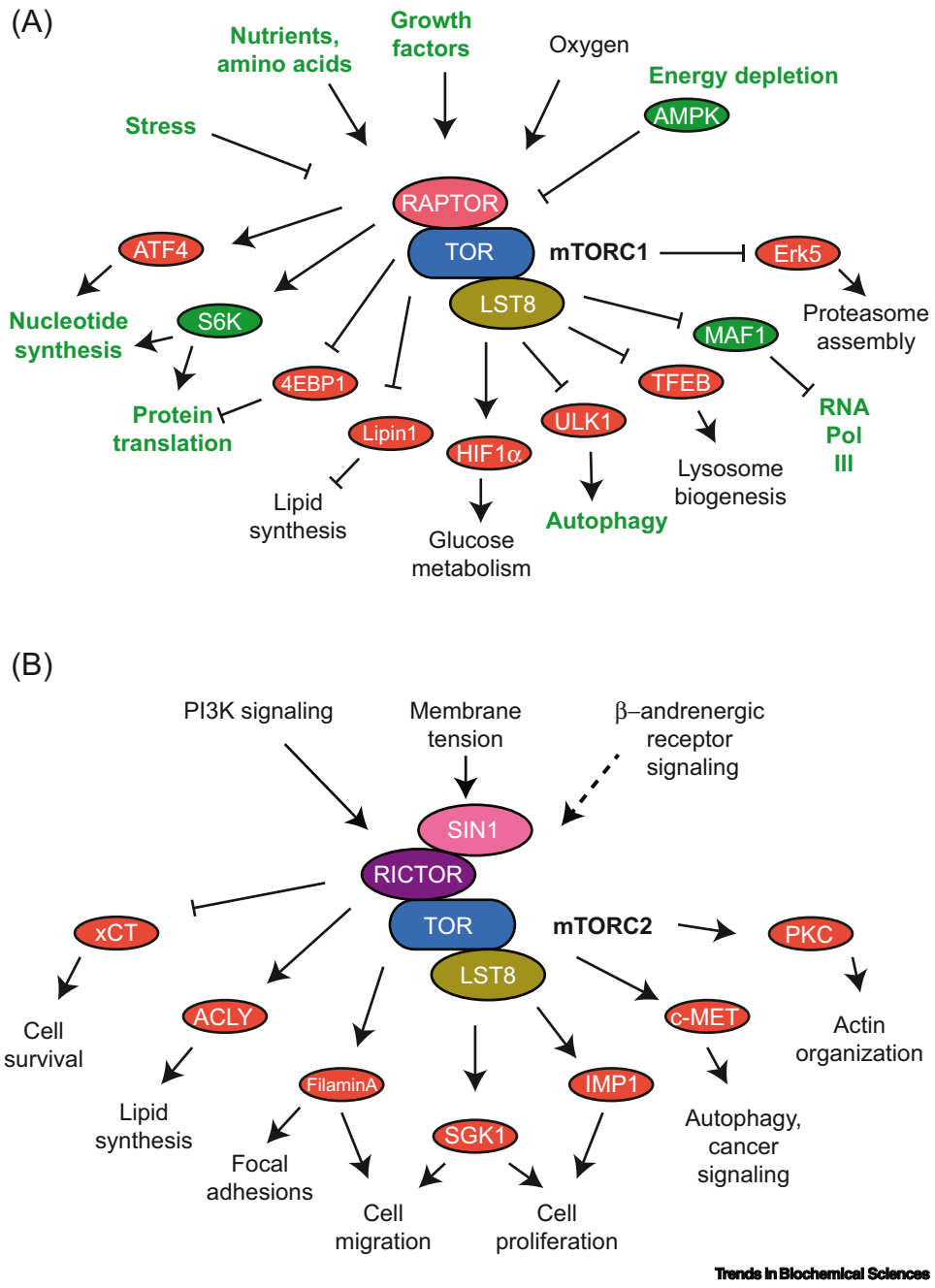

Figure 1. Overview of Mammalian Target of Rapamycin Complex (mTORC) Signaling. (A) Upstream activators/repressors and downstream effectors of mTORC1 regulate numerous metabolic and catabolic processes. Activators, effectors, and processes in green are conserved TOR-regulated processes in plants. (B) Signaling through mTORC2 modulates diverse cellular functions. Abbreviations: LST8, lethal with SEC13 protein 8; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; RAPTOR, regulatory-associated protein of mTOR; S6K1, S6 KINASE β-1; RICTOR, rapamycin-insensitive companion of mTOR; SIN1, SAPK-interacting protein 1

Glossary

Cryptochrome: photoreceptor proteins that are sensitive to blue light.
Galactolipids: dominant class of lipids in plastid membranes; these are essential for the function of photosynthesis.
Gravitropism: differential growth in response to gravity.
Infection thread: internal tubule formed within the root hair as rhizobia begin to infiltrate the plant during nodulation.
Lethal with SEC13 protein 8 (LST8): a WD-40 repeat containing protein found in both mTORC1 and mTORC2; serves as a scaffold for protein interaction.
Nodulation: process of forming nitrogen-fixing root nodules containing symbiotic bacteria (rhizobia) in members of the legume family.
P_{II} proteins: prokaryotic signal transduction proteins involved in nitrogen metabolism that are also found in plant plastids.
Phototropism: differential growth in response to light.
Phytochrome: photoreceptor proteins that respond to red and far-red light.
Pyrabactin: synthetic sulfonamide that mimics ABA.
Rapamycin-insensitive companion of mTOR (RICTOR): protein necessary for mTORC2 assembly.
Regulatory-associated protein of mTOR (RAPTOR): component of mTORC1 that binds to substrates of TOR, such as S6K.
SAPK-interacting protein 1 (SIN1): protein necessary for mTORC2 assembly.
Skotomorphogenic development: development of a seedling growing in the dark, characterized by a rapidly elongating hypocotyl and tight apical hook.
Thylakoid membranes: system of interconnected membranes within the chloroplast that carry out the light reactions of photosynthesis.
Type 2A associated phosphatase-associated protein 42 (TAP42): involved in the negative regulation of TOR signaling.
YET ANOTHER KINASE 1 (YAK1): member of the dual-specificity tyrosine phosphorylated kinase family.

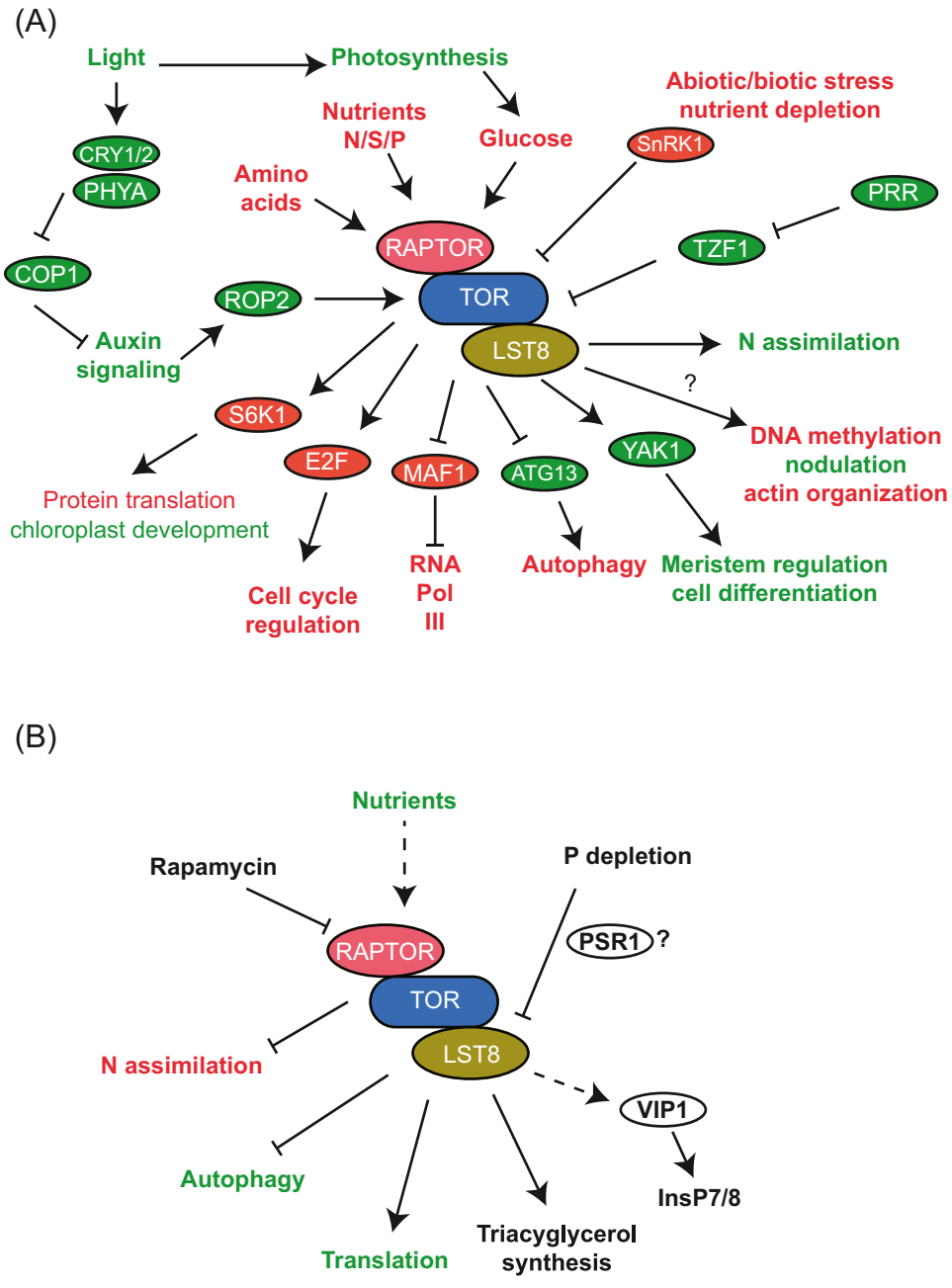

Figure 2. Summary of Plant Target of Rapamycin (TOR) Signaling in *Arabidopsis thaliana* and *Chlamydomonas reinhardtii*. (A) Inputs and mechanisms for the activation/inhibition of TOR signaling and the downstream processes regulated by TOR in *A. thaliana*. Activators/inhibitors, effectors, and cellular processes in red are conserved with mammalian TOR signaling. Those in green are plant specific. (B) Inputs and mechanisms for the activation/inhibition of TOR signaling and the downstream processes regulated by TOR in *C. reinhardtii*. Activators/inhibitors, effectors, and cellular processes in green are conserved with arabidopsis TOR signaling. Nitrogen assimilation in *C. reinhardtii* is highlighted in red because TOR appears to have the opposite regulation on this process compared with arabidopsis. Abbreviations: ATG, autophagy-related protein; COP1, CONSTITUTIVE PHOTOMORPHOGENESIS 1; LST8, lethal with sec13 protein 8; N, nitrogen; P, phosphorus; PRR, pseudo response regulators; PSR1, phosphate starvation regulator protein 1; RAPTOR, regulatory-associated protein of mTOR; ROP2, RHO OF PLANTS 2; S, sulfur; S6K1, S6 KINASE β-1; YAK1, yet another kinase 1.

pressure, membrane tension and homeostasis, actin cytoskeleton remodeling, and the pentose phosphate pathway [13,14]. While several TORC2-controlled processes are specific to metazoans, other processes that occur in yeast are relevant for plant growth and development, including cell wall integrity sensing, actin cytoskeleton organization, plasma membrane tension and homeostasis, and turgor pressure [11,13,14]. The absence of the canonical TORC2 components, namely RICTOR and SIN1, in plants suggests that either certain processes are controlled by other regulators, or that plant TOR is able to regulate the same processes via TORC1 or perhaps through a novel protein complex.

Our knowledge of TOR signaling in plants lags behind that in metazoans and yeast. While there are many similarities, plants lack homologs of some of the genes involved in signaling up or downstream of TOR. Plants also perform unique processes and being sessile organisms, face different environmental challenges to animals. Here, we present a broad overview of recent developments in plant TOR signaling, focusing on findings in the model alga and plant *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*, respectively, and present some pressing questions to be addressed as we move onward in the study of TOR signaling in plants.

A TOR Umbrella for the Signaling of Key Nutrients

Carbon (C), nitrogen (N), sulfur (S), and phosphorus (P) are essential nutrients for building key macromolecules. Little is known about how plants sense levels of C, N, S, and P, but evidence supports an involvement of TOR. C levels are sensed through the levels of photosynthates, such as glucose. The mechanisms by which glucose activates plant TOR signaling are not yet understood, but it has been established that glucose–TOR signaling activates genes involved in the biosynthesis of nucleotides, amino acids, proteins, and lipids, while repressing genes involved in the catabolism of these products (Figure 2A) (for an in-depth review, see [15]).

Rapamycin-mediated inhibition of TOR in *C. reinhardtii* leads to an increase in N assimilation, with a subsequent increase in amino acid production (Figure 2B) [16], while silencing of *TOR* or the TOR effector TYPE 2A PHOSPHATASE-ASSOCIATED PROTEIN of 46 KDA (*TAP46*) genes in arabidopsis results in downregulation of genes involved in N assimilation and increased expression of genes involved in N recycling [17,18], indicating that TOR signaling does have a role in regulating N uptake and incorporation but that role may differ between arabidopsis and *C. reinhardtii*. Plant TOR signaling has also been implicated in sensing available N, but the exact mechanisms remain unclear [19]. Known N-sensing mechanisms, such as the general control nonderepressible 2 (GCN2) pathway, sensing of glutamate by $\mathbf{P_{II}}$ plastidic proteins, and the family of glutamate receptor-like (GLR) proteins, are potential candidates to serve as an upstream N sensor for TOR signaling [19]. GCN2, a eukaryotic initiation factor 2α (eIF2α) kinase, has been implicated as a sensor for C and N precursors for cysteine biosynthesis [20].

Depletion of S precursors for cysteine biosynthesis through the mutation of sulfite reductase *sir1-1* inhibits TOR activity through the downregulation of glucose metabolism, resulting in decreases in protein translation, meristematic activity, and elevated autophagy [20]. Combining the *sir1-1* mutant with *cad2-1*, a cadmium-sensitive mutant that exhibits reduced glutathione synthesis, ameliorated some of the growth reduction observed in *sir1-1* plants and increased ribosome abundance when TOR was activated, indicating that TOR regulates the allocation of cysteine between glutathione and protein synthesis [21]. Such a mechanism of detecting cysteine precursors may enable plants to finely tune responses to limitations of specific nutrients.

In plants, whether TOR is also involved in P signaling and in the well-known role of P in organ growth [22] are yet to be established. In arabidopsis, the INOSITOL PENTAKISPHOSPHATE

2-KINASE (*AtIPK1*) gene has an important role in the regulation of phosphate homeostasis, because partial loss-of-function mutants have reduced levels of inositol hexakisphosphate ($InsP_6$) and altered expression of genes involved in Pi uptake, allocation, and starvation-response genes [23] In *C. reinhardtii*, phosphate starvation regulator protein 1 (PSR1) is a key transcription factor in P depletion conditions [24]. In response to P depletion, LST8 levels decrease, which results in lower TOR activity and reduced phosphorylation of the ribosomal protein S6 (RPS6), an established indirect downstream target of TOR signaling. By contrast, in *psr1* mutants, LST8 levels do not decrease in P-deplete conditions and RPS6 phosphorylation is increased [25]. PHOSPHATE STARVATION RESPONSE 1 (PHR1) in arabidopsis belongs to the same family of transcription factors as PSR1 in *C. reinhardtii* [26]. Although it is not as responsive to Pi starvation as PSR1, further investigation to determine whether PHR1 is involved in TOR signaling could be worthwhile. Mutation of VIP1, an inositol hexakisphosphate kinase in *C. reinhardtii*, displays increased sensitivity to rapamycin, and reduced levels of the signaling molecules inositol phosphates $InsP_7$ and $InsP_8$ [27]. These results imply that PSR1 is involved in TOR signaling for P availability, possibly through InsP signaling, at least in algae; however, the mechanisms that connect PSR1 to changes in TOR are yet unknown (Figure 2B).

In all, the available evidence to date hints at a functional connection between nutrient sensing and TOR activity, but the underlying mechanisms are yet to be determined. Some such mechanisms may be direct or indirect (i.e., signaling for one nutrient may share or impact signaling components for a different nutrient), underscoring that, as key nutrient signaling and monitoring proteins are identified or studied in the context of TOR signaling, it will be critical to establish how and if they relay information directly to TOR.

Amino Acid Signaling and Homeostasis Controlled by TOR

Similar to mammals and yeast, amino acids (AAs) are one of the many inputs that stimulate TOR activity to promote growth in plants (Figure 2A) [8]. However, plants lack the mammalian and yeast RAG GTPases and Ragulator complex responsible for AA sensing [6,10]. Thus, the mechanisms for AA sensing in plants are unknown, but several recent studies have started to elaborate the effects of particular AAs on TOR signaling. For example, sufficiently high levels of proline and alanine promote the activation of TOR. This in turn downregulates mitochondrial pathways that consume AAs for respiration and upregulates protein synthesis [28]. Loss-of-function mutations of ISOPROPYLMATAE SYNTHASE 1 (IPMS1), a leucine biosynthetic gene, led to increased accumulation of branched-chain AAs (BCAAs), most notably valine [29,30]. This resulted in activation of TOR leading to a variety of defects in plant growth and development, such as an increase in the number of meristematic cells that were smaller and defects in chloroplast development compared with wild type [29,30]. Additional work is needed to determine whether levels of specific AAs or AA biosynthetic precursors serve as identifiers of nutrient levels and how this information is incorporated into TOR signaling. Interestingly, increased TOR activity due to high levels of BCAAs resulted in a higher degree of actin bundling, which was repressed by inducible RNAi of TOR (Figure 2A) [29]. Furthermore, BCAA treatment was still able to induce actin bundling in *raptor1b* mutants, suggesting that this occurs in a TORC1-independent manner [29]. Indeed, because actin organization is dependent on TORC2 in yeast and metazoans, these findings imply that, while the canonical TORC2 components are absent in plants, TOR is able to regulate the actin cytoskeleton in a manner unique to plants [29].

TOR Influences Symbiotic Relationships

Plants have the ability to form symbiotic relationships with soil-dwelling bacteria. Given that these relationships involve nutrient exchange between plants and bacteria as well as organ development and growth, perhaps a role of TOR signaling in the establishment of symbiotic relationships

is not entirely surprising. Indeed, TOR expression increased during **nodulation** of bean (*Phaseolus vulgaris*) and decreased TOR activity impaired **infection thread** development and altered nodule development [31]. Consistently, by producing auxin, the plant growth-promoting rhizobacterium *Azospirillum brasilense* increases TOR expression in the root meristem, stimulating root meristem cell division and lateral root formation [32]. One interesting process to look at in the future is the role of TOR in sensing N availability in relation to nodule development, which is inhibited when N availability is nonlimiting [33].

Plant Meristem Regulation, Cell Differentiation, and an Unexpected Connection with the Circadian Clock

The apical meristems are key sources of stem cells, which can elongate to lengthen the shoot or root, or differentiate for organogenesis. TOR has a critical role in the proper maintenance of both root and shoot apical meristems, but the requirements for TOR activation in each are different. Glucose signaling is sufficient for activation of TOR in the root meristem; however, both glucose and light are required for TOR activation in the shoot meristem and for the transition to photoautotropic growth (Figure 2A) [34]. A picture of the components of TOR signaling in plants has recently emerged. Treatment with the TOR inhibitor AZD8055 reduced root meristem cell number and induced early cell differentiation. Loss-of-function mutants of **yet another kinase 1 (YAK1)**, a downstream effector of TOR, are resistant to AZD8055, while YAK1 overexpression confers hypersensitivity to AZD8055 [35]. When TOR activity was inhibited, YAK1 activated Siamese-related (SMR) cyclin-dependent kinase inhibitors to induce cell differentiation (Figure 2A) [35]. Interestingly, TOR also has a role in cell dedifferentiation. For instance, TOR phosphorylation and stabilization of the transcription factor E2Fa is critical for sugar-induced callus formation from differentiated tissue through the activation of S-phase genes necessary for cell cycle progression (Figure 2A) [36].

Links between meristem regulation through TOR signaling and the circadian clock have recently been uncovered. Members of the circadian-signaling pseudo response regulators (PRR) family inhibit TANDEM ZINC FINGER 1 (TZF1), which binds to and affects the stability of TOR mRNA to represses TOR signaling-mediated root cell proliferation (Figure 2A) [37]. Furthermore, nicotinamide blocks glucose–TOR signaling effects on root meristem activation, root growth, and circadian period adjustment by inhibiting ATP production [38]. Similarly, chemical inhibition of TOR or knockdown of TOR transcripts lengthened the circadian period, similar to that observed during chemical inhibition of the electron transport chain [39]. These findings support that TOR signaling uses ATP sensing as an input to modulate the circadian period and plant growth.

The Emergent Role of TOR in Plant Autophagy

Autophagy enables organisms to recycle cellular components and, in plants, is upregulated in conditions of nutrient starvation and other growth-limiting stresses [40]. TOR signaling inhibits autophagy through pathways that are now emerging in plants. In arabidopsis, SUCROSE NONFERMENTING-RELATED PROTEIN KINASE 1 (SnRK1) acts upstream of TOR to repress TOR activity and promote autophagy (Figure 2A) [41]. This is likely executed via the SnRK1 subunit KIN10. Indeed, overexpression of KIN10 led to elevated autophagy levels in stress conditions, while *kin10* mutant plants failed to activate autophagy under similar conditions [41]. Conversely, overexpression of TOR inhibited activation of autophagy under starvation, salt, and osmotic stresses, but not oxidative or endoplasmic reticulum (ER) stress [42]. Similar results were observed when exogenous auxin was applied to activate TOR signaling [42].

Insights into the TOR-mediated inhibitory mechanisms of autophagy were provided by an analysis of ATG13, one of the members of the largely conserved autophagy-related proteins (ATG) that

are necessary for various steps in autophagy. Similar to other RAPTOR-interacting proteins, such as ribosomal protein S6 KINASE β-1 (S6K1), and PROTEIN PHOSPHATASE 2A/B (PP2A/B), ATG13 contains a conserved five-AA motif, which was required for interaction with RAPTOR and subsequent phosphorylation by TOR [43]. Similar to yeast, hyperphosphorylated forms of ATG13 are found in nutrient-rich conditions, whereas, in nutrient-limiting conditions, the ATG1/13 complex is degraded through autophagy [44,45]. TOR phosphorylation of ATG13 in arabidopsis appears to have a similar inhibitory effect on autophagy, as supported by evidence that deletion of this motif from ATG13 in arabidopsis led to elevated levels of autophagy (Figure 2A) [43]. In *C. reinhardtii*, TOR inhibition by rapamycin treatment led to elevated levels of ATG8, similar to the effects of oxidative stress, ER stress, and C/N-limiting conditions, and is indicative of the activation of autophagy [46]. These results illustrate a feedback loop between TOR signaling and autophagy to increase the recycling of key resources in times of stress through modulation of the activity of proteins directly involved in autophagy.

Photosynthesis: A Multilayered Involvement of TOR

Photosynthesis is one of the most important processes in plants, producing sugars from carbon dioxide, water, and sunlight. TOR appears to be involved in several steps preceding photoautotrophy and maintenance of photosynthesis. As discussed earlier, both glucose and light-induced auxin biosynthesis are required for TOR activation in the shoot meristem to facilitate conversion to photoautotropic growth in arabidopsis [34]. Mechanistically, CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) inhibits TOR activity during **skotomorphogenic development** of etiolated seedlings. Light perception by **phytochrome** A and **cryptochrome** inactivate COP1 to promote the auxin-mediated activation of TOR and the downstream phosphorylation of RPS6, enhancing protein translation in de-etiolating seedlings to promote cotyledon opening and the transition to photoautotrophic growth (Figure 2A) [47].

TOR signaling also induces expression of genes involved in chloroplasts and photosynthesis, while TOR inhibitors have been shown to reduce chloroplast number and size and, thus, impair photoautotrophic growth [48,49]. In rice, suppression of *s6k1* and/or *raptor2* yielded pale yellow-green leaves and altered **thylakoid membrane** structure, and further investigation demonstrated that S6K1 signaling is vital for **galactolipid** biosynthesis for the thylakoid membrane and fatty acid homeostasis (Figure 2A) [50].

Glucose production in leaves promotes TOR activity and, thus, TOR activity is higher in mature source leaves, while developing sink leaves that still do not produce sufficient glucose leaves have lower TOR activity [51]. Higher TOR activity facilitates loading glucose into the phloem through plasmodesmata, channels between cells that allow the exchange of cytoplasmic contents, enabling the transport of sugars from source to sink leaves [51].

An examination in *C. reinhardtii* of proteins that exhibited changes in oxidation during TOR inhibition revealed a reduction in photosynthetic electron flow and identified 20 proteins involved in photosynthesis with altered oxidation status [52]. The reduction in electron flow during inhibition of TOR was also observed in another study that also found that TOR inhibition caused altered chloroplast morphology and increased non-photochemical quenching and damage to photosystem II reaction centers [53]. In this same study, it was observed that inhibition of TOR also led to an increase in fragmentation of mitochondria and increased mitochondrial respiration, suggesting that TOR balances chloroplast and mitochondria functions in algae [53].

While research has demonstrated that TOR signaling is important for the initiation of photoautotropic growth and the photosynthetic machinery, what is not known is whether any

other signals besides glucose allow TOR to sense and regulate photosynthetic processes once established.

TOR Signaling and Phytohormones

Phytohormones are small signaling molecules that are involved in developmental processes and responses to external stimuli [54]. In relation to TOR activity, these can be separated into two groups based on whether they promote or inhibit growth (Figure 3). When TOR activity is inhibited, the genes involved in the signaling pathways of phytohormones that promote growth (auxin, gibberellic acid, brassinosteroids, and cytokinins) are repressed, while those that inhibit growth [abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), and ethylene] are upregulated [48]. Some of these changes in gene expression may result from changes in DNA methylation, because a new study has shown that inhibition of TOR resulted in decreased methylation of the genome and many of the differentially methylated genes were involved in plant hormone signaling and metabolic processes [55]. An involvement of arabidopsis MAF1, a conserved stress-responsive Pol III repressor that is regulated by TOR [56], is also plausible but yet untested.

Here, we report some recent discoveries about the interaction between phytohormone and TOR signaling.

Growth-Promoting Phytohormones

Auxin and its analogs are crucial for plant growth by regulating cell division in the shoot and root meristems, elongation of maturing cells, cell differentiation, and growth phenomena, such as

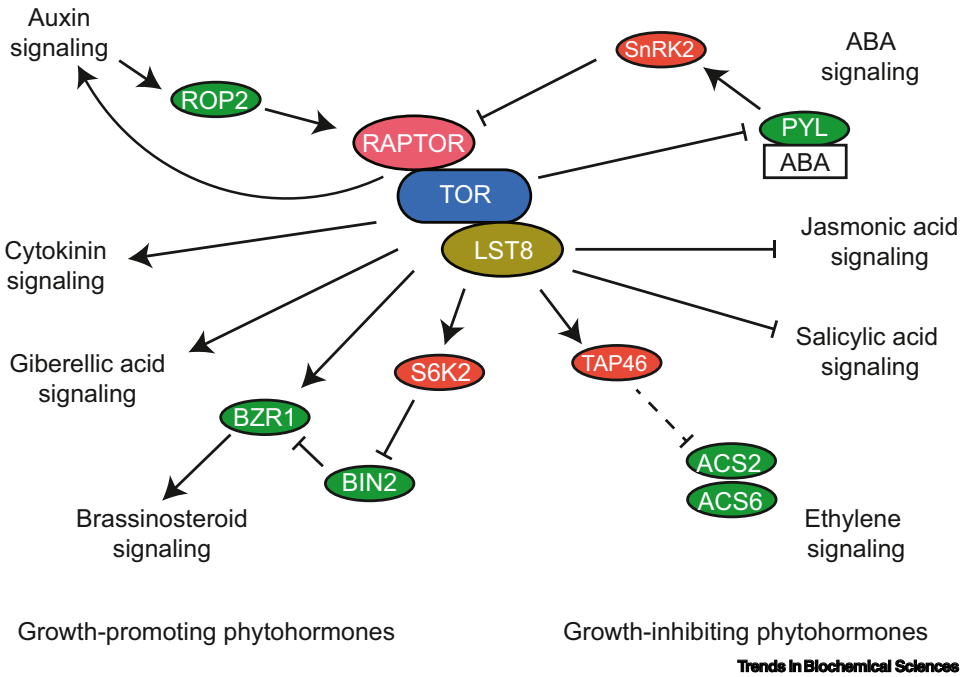

Figure 3. Overview of Interaction between Target of Rapamycin (TOR) Signaling and Phytohormones in *Arabidopsis thaliana*. Proteins in red are conserved regulators or effectors of TOR signaling in the mammalian system. Abbreviations: ABA, abscisic acid; ACS, AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE; BIN2, BRASSINOSTEROID INSENSITIVE 2; BZR1, BRASSINAZOLE RESISTANT 1; LST8, LETHAL WITH SEC13 PROTEIN 8; PYL, PYRABACTIN-LIKE; RAPTOR, REGULATORY-ASSOCIATED PROTEIN OF mTOR; ROP2, RHO OF PLANTS 2; S6K2, S6 KINASE β-2; SnRK2, SUCROSE NONFERMENTING-RELATED PROTEIN KINASE 2; TAP46, TYPE 2A PHOSPHATASE-ASSOCIATED PROTEIN of 46 KDA.

gravitropism and **phototropism** [57]. The plant responses to auxin treatments are attenuated in the presence of TOR inhibitors, causing shorter primary roots, a reduced number of lateral roots, and a lack of gravitropism compared with plants treated with auxin alone [58]. When activated by exogenous auxin, the small GTPase RHO OF PLANTS 2 (ROP2) binds and activates TOR and, through S6K1, promotes translation re-initiation at upstream open reading frames of genes involved in the regulation of stems cells and cell differentiation (Figure 3) [59,60]. Hence, ROP2 may be a component of the auxin–TOR integrating machinery for the control of cell differentiation and growth.

Brassinosteroids contribute to the regulation of many cellular processes, including cell division and elongation, stress responses, and photomorphogenesis [61]. Chemical or genetic inhibition of TOR activity reduced the levels of BRASSINAZOLE RESISTANT 1 (BZR1), a brassinosteroid signaling transcription factor that promotes growth and the expression of brassinosteroid-responsive genes [62]. BZR1 is destabilized via phosphorylation by BRASSINOSTEROID INSENSITIVE 2 (BIN2), which was found to be a target of TOR through the ribosomal protein S6K2, indicating that TOR–S6K2 signaling promotes the accumulation of BZR1 to facilitate this transition from heterotrophic to photoautotrophic growth (Figure 3) [49,63].

These findings uncover the existence of positive regulators of growth that respond to TOR. Future work may identify the growth-ceasing factors connected with TOR signaling that supersede the positive role of endogenous auxin and brassinosteroids to halt growth during development.

Growth-Inhibiting Phytohormones

ABA is a key regulator of plant responses to abiotic stresses, such as drought, and is also important for seed development and germination [64]. In the absence of stress, TOR signaling phosphorylates ABA receptors of the **pyrabactin**-like (PYL) protein family to inhibit ABA signaling (Figure 3) [65]. Conversely, in response to stress, ABA levels rise; ABA binds to PYL and inhibits PP2C proteins, allowing the release of SnRK2 from the complex; SnRK2 then phosphorylates RAPTOR to inhibit TOR activity and growth (Figure 3) [65]. Inhibition of TOR or mutations in LST8-1 or RAPTOR led to decreased ABA levels due to reduced expression of ABA biosynthetic genes, as well as an increased sensitivity to ABA in seed germination and plant growth [66]. Mutations in YAK1 suppressed hypersensitivity to ABA in *lst8-1* mutants [67]. In mammalian and yeast cells, **type 2A associated phosphatase-associated protein 42 (TAP42)** an interacting protein of 41 kDa (TIP41) was shown to be a downstream effector of TOR interacting with PP2A, which facilitates multiple ABA-regulated processes in plants. Consistently, *tip41* mutant plants are smaller and hypersensitive to both TOR inhibitors and ABA [68,69]. Together, these discoveries highlight not only a regulatory loop between ABA and TOR signaling, but also that multiple factors may contribute to the antagonistic interactions between TOR and ABA signaling. These are likely needed to tune the ability of plants to control growth in response to the type and entity of environmental stresses.

JA has a role in numerous plant growth and developmental processes, but may be best known for its role in plant responses to abiotic and biotic stresses, such as wounding and challenge by pathogens [70]. Recent developments have started to uncover the antagonistic relationship between JA and TOR signaling. Compared with wild type, *raptor1b* mutants exhibit constitutively reduced JA levels in leaves. Nonetheless, upon wounding, these mutants can produce JA. Furthermore, treatment with the TOR inhibitor AZD8055 induced high levels of JA in both wild type and *raptor1b* mutants [71]. Similarly, AZD8055 combined with methyljasmonate inhibited plant growth, indicating crosstalk between TOR and JA signaling [71,72]. In support of this,

similar to the antagonistic relationship found between TOR activity and ABA response, plants with increased TOR activity were more vulnerable to pathogens, while TOR repression yielded smaller, but more resistant plants [73]. Further investigation may uncover the mechanism for how JA can inhibit TOR signaling to shift from growth to defense, perhaps through a regulatory loop similar to that found for ABA.

Ethylene is a key phytohormone in the processes of ripening, leaf senescence, and abscission, as well as plant growth and development, and stress responses [74]. Similar to ABA and JA, inhibition of TOR increased the expression of genes involved in senescence, ethylene signaling, and biosynthesis [68]. Furthermore, ethylene-insensitive mutants displayed a reduced sensitivity to AZD8055 and ethylene signaling reduced the inhibition of hypocotyl growth in AZD8055-treated seedlings [75]. TAP46 interacts with the ethylene biosynthesis pathway proteins AMINOCYCLOPROPANE-1-CARBOXYLATE SYNTHASE 2 (ACS2) and ACS6, which accumulate when TOR is inhibited, suggesting that TOR regulates ethylene signaling through modulation of ethylene production [68]. These findings underscore an antagonistic growth regulation by ethylene and TOR. It is possible that ethylene signaling may be also involved in inhibiting TOR signaling during senescence, and perhaps future research can uncover the underlying mechanisms.

Concluding Remarks

Compared with noticeable progress in the study of TOR signaling mechanisms in mammalian and yeast systems, our understanding of plant and algal TOR has many more gaps (see Outstanding Questions). Notably, while addition of individual AA has been shown to stimulate TOR activity, the mechanisms by which plants sense AA are unknown. Similarly, identifying the sensing mechanisms for essential nutrients and how these impact TOR signaling will be crucial for our understanding of how the processing of multiple inputs factors into plant growth. This knowledge may allow for a more in-depth examination of plant symbiosis with rhizobium and mycorrhizae. Understanding the crosstalk between phytohormone and TOR signaling pathways will allow for identifying key growth-promoting and growth-ceasing factors that direct plant growth and development, as well as stress responses. While plants lack the RICTOR and SIN1 components to form TORC2, many of those functions regulated by TORC2 in yeast, such as membrane tension, turgor pressure, cell wall integrity, and actin organization, are also crucial for plants as well. While there is evidence that TOR activity impacts the actin cytoskeleton, it remains to be seen whether these other processes are regulated by TOR and, if so, whether it is done through TORC1 signaling or some novel, plant-specific mechanism. Lastly, while the role of TOR has been established in chloroplast development and the inhibition of TOR has been shown to impact photosynthesis, it appears that the status of such a crucial process would likely be sensed by TOR through more than just the production of sugars. Whether TOR activity is responsive to processes such as photodamage or photoinhibition, or is involved in chloroplast repositioning during changes in light intensity, also need to be investigated.

Several mechanisms and functions attributed to TOR appear to have plant-specific features. While this makes it difficult to translate knowledge of TOR signaling in non-plant species to plants, it is also exciting. This is because we may be able to identify new pathways in plant TOR, which may eventually reveal convergent features with non-plant TOR signaling. For example, the involvement of plant TOR in symbiosis may reveal foundational mechanisms underpinning the establishment and maintenance of the microbiome in mammals. Above all, the availability of viable mutants in plant TOR signaling and the possibility to generate whole higher-order mutants of TOR with disrupted signaling pathways will be invaluable to query mechanisms for conserved processes with metazoans (e.g., cell growth and proliferation, or organ elongation).

Outstanding Questions

How do plants monitor the availability of nutrients and how is that information relayed to TOR?

How are amino acid levels sensed in plants and how is that information relayed to TOR?

Do plants still utilize TOR to regulate cellular processes traditionally mediated by TORC2?

How do phytohormone signaling and TOR signaling pathways interact with each other?

Does a mechanism exist for TOR to monitor the photosynthetic apparatus?

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