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Plant TOR signaling components

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Cell growth is a process that needs to be tightly regulated. Cells must be able to sense environmental factors like nutrient abundance, the energy level or stress signals and coordinate growth accordingly. The target of rapamycin (TOR) pathway is a major controller of growth-related processes in all eukaryotes. If environmental conditions are favorable, the TOR pathway promotes cell and organ growth and restrains catabolic processes like autophagy. Rapamycin is a specific inhibitor of the TOR kinase and acts as a potent inhibitor of TOR signaling. As a consequence, interfering with TOR signaling has a strong impact on plant development. This review summarizes the progress in the understanding of the biological significance and the functional analysis of the TOR pathway in plants.

The TOR kinase acts as a central component of TOR signaling and modifies several downstream proteins by phosphorylation. TOR is part of two distinct multi protein complexes, namely TORC1 and TORC2, which are controlling diverse cellular processes such as autophagy, protein translation, ribosome biogenesis, and actin dynamics.[1] Mitochondrial oxidative function, which impacts aging processes, and the production of reactive oxygen species (ROS) are also strongly influenced by TOR signaling.[2] ROS can have cytotoxic effects but also possess a very important function as signaling molecules in diverse cellular processes. In plants, these molecules have been shown to be important for pathogen defense, polar cell growth, and the remodeling of the cell wall.[3]

The name of the TOR kinase and the entire pathway describes a characteristic property—the specific and effective inhibition by rapamycin, a macrocyclic lactone of bacterial origin. This sensitivity makes rapamycin a drug with very interesting properties for clinical and basic research.[4-6] Its potential is already used in tumor treatment, cardiology, transplantation medicine and treatment of neuronal diseases.[7-9]

Structure of the TOR Protein

The TOR protein belongs to the family of PIKK (phosphatidylinositol kinase-related kinases) which represent a group of conserved serine/threonine kinases. In addition to the kinase domain, the TOR protein possesses further distinct domains. In the N-terminal region, TOR consists of up to 20 tandem HEAT repeats (Huntingtin, elongation factor 3 [**E**F3], protein phosphatase 2A [PP2**A**], yeast PI3-kinase **T**OR1), followed by the FAT domain (FRAP/ATM/TRRAP), the FRB domain, the kinase domain and the FATC domain (**Fig. 1**). The latter four domains are found in all PIKKs and thus seem important for the activity of this class of kinases. The HEAT repeats have been shown to mediate protein-protein interactions and are found in several cytoplasmic proteins including the four giving rise to the acronym.[10] The inhibition of TOR by rapamycin requires the formation of a ternary complex of rapamycin, the peptidyl-prolyl cis/trans isomerase FKBP12, and the FRB (FKBP12 rapamycin binding) domain.[1,4,11] The redox state of the FATC domain seems to impact the degradation of TOR.[12] Yet, Ren and workers have shown that in *Arabidopsis thaliana*, the FATC domain is not essential for TOR function.[13] For a detailed structural analysis of TOR, see Knutson.[10]

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Plant TOR Proteins

TOR kinases from very diverse eukaryotic species show a high degree of conservation in the kinase, FATC and the FRB domain but only to a limited extend in the number of HEAT repeats. As for most eukaryotes, the plant model species *Arabidopsis thaliana* possesses a single *TOR* gene coding for a protein of approximately 280 kDa.[14] Maize (*Zea mays*) is the only other plant for which the TOR kinase has been described. As for Arabidopsis, the maize TOR protein is encoded by one gene and is also comparable in size to the Arabidopsis TOR protein.[15] The closest homologs of the Arabidopsis TOR are the TOR proteins of *Populus trichocarpa* (identity: 82%, similarity: 89%) and the TOR protein isoforms one (identity: 80%, similarity: 88%) and two (identity: 79%, similarity: 87%) of *Vitis vinifera*. In all these plant proteins, the general domain structure as well as the protein sequence is well conserved. The phylogenetic tree of TOR proteins from different species perfectly reflects phylogenetic relationships of the species. Four main groups are visible corresponding to the animal kingdom, fungi, algae and higher plants, respectively (**Fig. 2**). This strong conservation of TOR proteins throughout the species points out the general importance of this kinase and, consequently, the entire TOR pathway.

Sensitivity to Rapamycin

TOR activity can be inhibited by direct interaction of rapamycin with the cis/trans isomerase FKBP12 and the FRB domain of the TOR protein.[5] In animals, TOR is sensitive to rapamycin. Also

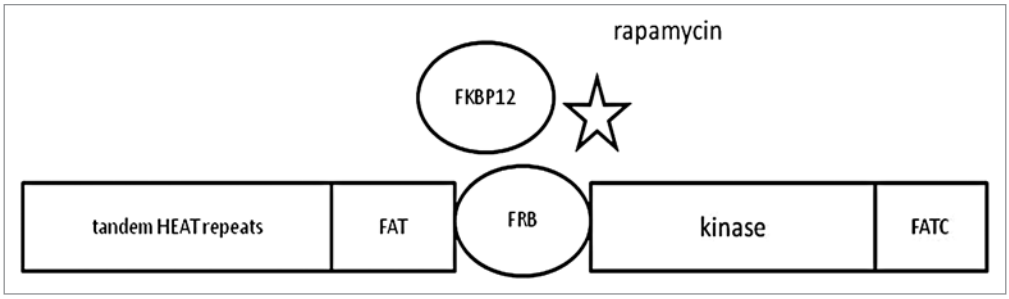

Figure 1. Structure of the TOR protein. The TOR protein belongs to the family of PIKK (phosphatidylinositol kinase-related kinases). In the N-terminal region, TOR proteins possess up to 20 tandem HEAT repeats which have been shown to mediate protein-protein interactions. The catalytic domain is flanked at the N-terminus by the FAT (FRAP/ATM/TRRAP) and the FRB domain, the latter being the binding site of the TOR-inhibiting drug rapamycin and the peptidyl-prolyl cis/trans isomerase FKBP12. C-terminal of the kinase domain is the FATC domain which seems to influence TOR turnover rate in response to the redox state of the cell.

for maize and the unicellular algae *Chlamydomonas reinhardtii,* rapamycin sensitivity was demonstrated.[16,17] Some land plants, however, lost this sensitivity. Among these plants are Arabidopsis and *Vicia faba*.[18] In Arabidopsis the sensitivity to rapamycin can be restored by the expression of *FKBP12* of *S. cerevisiae*. These lines display a reduction in primary root lengths, epidermal cell lengths, less polysome accumulation and an overall reduction of growth after treatment with rapamycin.[19,20]

Functional Importance of the TOR Kinase in Plants

Both in plants and animals, TOR exerts a very general function in anabolic and catabolic processes as described above. In Arabidopsis, a promotor-*GUS* fusion construct revealed *TOR* expression throughout early development in the endosperm, the embryo and the chalazal proliferating tissue. After the early globular stage, *TOR* is no longer expressed in the endosperm but persists in the embryo up to the heart and torpedo stages. In both the seedling and adult plant, *TOR* expression can be detected to a high level in the primary meristems. This suggests that *TOR* expression in Arabidopsis is predominant in zones where cell proliferation is coupled to cytosolic growth, which would be in contrast to mammalian cells and Drosophila were *TOR* expression occurs in all tissues.[14,21,22] Microarray data, however, suggest that a basal level of *TOR* expression is found in all Arabidopsis tissues.[23] In maize, *TOR* expression has been shown to begin during germination at approximately 12 h and increases to the highest level at 48 h. Also, *TOR* RNA has been shown to be present in in all tissues of 13 d-old seedlings at almost the same level, regardless of their developmental stage.[15]

Elucidating TOR function in Arabidopsis by mutations is hampered by the fact that a *TOR* knockout mutant shows an embryo-lethal phenotype and arrests endosperm and embryo development at a premature stage.[14] Thus, a further functional characterization effort was performed using an ethanol-inducible RNAi system. After *TOR* silencing was induced, the treated plants showed several severe growth defects. On the one hand, they almost completely stopped growth of existing leaves and on the other hand, the silenced plants showed symptoms which are usually linked to plant senescence such as early yellowing due to chlorophyll breakdown, accumulation of soluble sugars to a very high degree in the leaves, and a 2–3 fold higher glutamine synthetase and glutamate dehydrogenase activity.[24] Under stress conditions or during senescence, plant cells are recycling cytoplasmic content. This process is called autophagy and has been shown to be influenced by TOR signaling.[25] In Arabidopsis it could be shown that RNAi-*TOR* plants had constitutive autophagy and that some genes required for autophagy were upregulated.[26] Silencing of *TOR* has also an impact on the efficiency in mRNA translation, reflected by a reduction in the abundance of high molecular weight polysomes and a decrease in the amount of soluble protein. A higher level of *TOR* mRNA, instead, causes enhanced organ growth and a higher seed production.[24] In Arabidopsis, plants exposed to osmotic stress develop shorter primary roots. If *TOR* is constitutively expressed in Arabidopsis, this effect is alleviated.[24] These findings suggest that also in plants, TOR is influencing anabolic and catabolic growth processes as well as aging and nutrient recycling and seems to render plants more stress-resistant.

TOR-Binding Proteins

In mammals and yeast, TOR forms two multiprotein complexes TORC1 and TORC2, which contain at least TOR, mLST8, RAPTOR (regulatory associated protein of TOR) and TOR, mLST8, RICTOR, respectively.[1] RAPTOR functions in recruiting TOR substrate proteins and Arabidopsis encodes two RAPTOR proteins, *RAPTOR1A* and *RAPTOR1B*.[27] Homozygous knockout mutants of *raptor1a* do not show any visible mutant phenotype whereas homozygous *raptor1b* mutants exhibit a strong growth phenotype with slower growing roots which are thicker than the wild-type, coiled, and densely covered with root hairs. The phenotype appears to be caused by reduced meristematic activity, which is even further reduced in a *raptor1a raptor1b* double mutant.[28,29] In summary, the disturbance of the Arabidopsis TORC1 multiprotein complex leads to growth defects, emphasizing the importance of the TOR pathway as a growth control mechanism also in plants. Additional components of TORC1 as well as those of TORC2 remain to be analyzed.

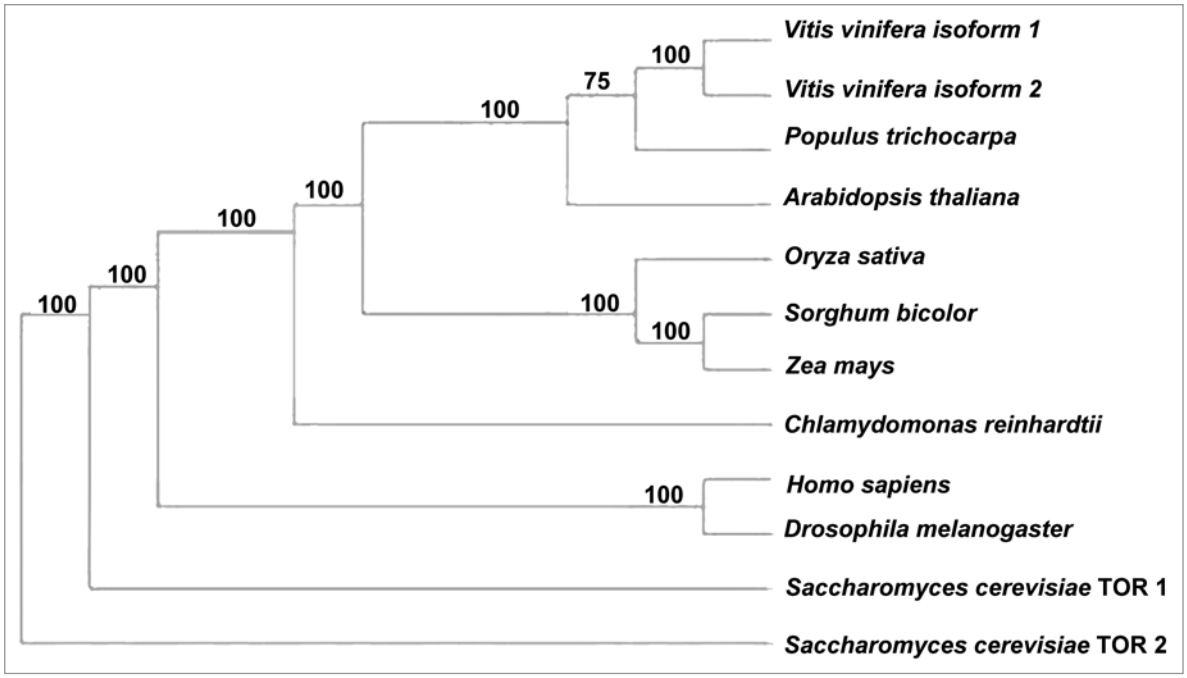

Figure 2. Phylogenetic tree of TOR proteins from different species. Four main groups are formed by the animal kingdom (H.s., D.m.), fungi (S.c.), algae (C.r.) and higher plants. The latter group can be subdivided into grasses (Z.m., S.b., O.s.) and dicotyledons (A.t., V.v., P.t.). The closest homologs of the Arabidopsis TOR are the TOR proteins of *Populus trichocarpa* and the TOR protein isoforms one (identity: 80%, similarity: 88%) and two (identity: 79%, similarity: 87%) of *Vitis vinifera*. In all these plant proteins, the general domain structure as well as the protein sequence is well conserved. The phylogenetic tree was done with the PHYLIP software and is based on a ClustalW multiple alignment of protein sequences. The bootstrap numbers indicate the number of times the group consisting of the protein sequences which are to the right of that fork occurred among the trees, out of 100 trees. All bootstrap values are high, indicating a very robust phylogenetic tree.

TOR Regulatory Network

A number of components of the TOR signaling pathway have been identified, revealing a highly complex network (**Fig. 3**). In mammals, activity of TOR is basically influenced by three major factors, the abundance of insulin-like growth factors, nutrients, as well as the cellular energy status. Changes in these conditions eventually lead to a modification of the tuberous sclerosis complex (TSC) which is a dimer of TSC1 (HAMARTIN) and TSC2 (TUBERIN) and a negative regulator of TOR. Growth factors act through the PI3K pathway and lead to activation of the kinases PDK1 and AKT. Activated AKT inhibits the suppressing action of TSC and thereby activates TOR. The nutrient status of the cell is particularly represented by the abundance of amino acids. The exact mechanism by which nutrient availability is monitored remains to be elucidated. It has been shown, however, that TORC1 is able to sense the cellular energy status through the AMP-activated protein kinase (AMPK).[1]

In plants, alternative signaling pathways must have developed since they seem to lack homologs of TSC1 and TSC2. Yet, some mechanisms appear conserved since homologs of the mammalian PI3K pathway components have been identified in maize. A 20 kDa insulin-related peptide (IGF) was isolated which has been shown to share epitope homology with mammalian insulin. The time of *IGF* expression coincides with the onset of fast growth during germination.[30] When maize seeds were treated with isolated IGF, a significant increment in coleoptile and root lengths could be detected in comparison with the untreated control. Furthermore, IGF and insulin stimulated selectively the translation of ribosomal proteins and led to a selective recruitment of translation apparatus mRNAs into polysomes. These effects could be blocked by treatment with rapamycin, suggesting the involvement of TOR signaling in the response to IGF or insulin.[17]

The translationally controlled tumor protein (TCTP) is an important component of the TOR signaling network. A large number of studies in various organisms have related TCTP to diverse cellular processes such as apoptosis, microtubule organization or ion homeostasis and several interacting proteins (e.g., Polo Kinase, Tubulin and Na^+/K^+-ATPase) were identified. TCTP is a guanine exchange factor of the small GTPase Rheb which has been shown to influence TOR in Drosophila. The Arabidopsis genome also codes for a TCTP. A knockout of *TCTP* leads to a male gametophytic phenotype with normal pollen formation and germination but impaired pollen tube growth, explaining the inability to find homozygous *tctp* mutants. Silencing of *TCTP* by RNA interference slows vegetative growth and leaf expansion is reduced due to a smaller cell size. Lateral root formation is reduced and root hair development is impaired. These lines also show decreased sensitivity to an exogenously applied auxin analog and have elevated levels of endogenous auxin.[31] Auxins are important plant growth stimulating hormones and TCTP seems to represent a link between these general growth promoting factors and TOR signaling.

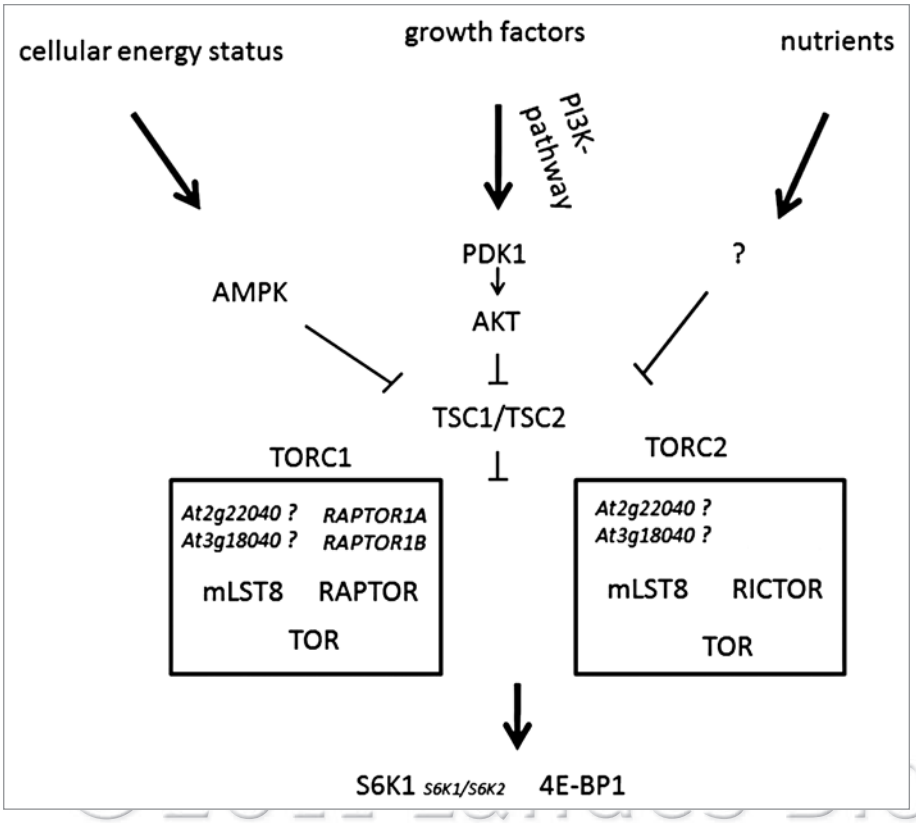

Figure 3. Simplified TOR signaling network with known Arabidopsis homologs in Italic writing. Activity of TOR is influenced by abundance of growth factors and nutrients as well as by the cellular energy level. Growth factors act through the PI3K pathway which finally leads to inactivation of the TOR inhibitory complex TSC1/TSC2. The cellular energy status is mostly represented by the abundance of amino acids which lead to the activation of AMPK kinase and to subsequent inactivation of TSC1/TSC2. How nutrient perception and intracellular signaling works in detail still remains to be elucidated. Gene identifiers represent possible Arabidopsis LST8 homologs.

Downstream Targets of TOR

The best studied downstream targets of TOR are the regulators of protein biosynthesis S6K1 (protein S6 kinase 1) and 4E-BP1 [eukaryotic initiation factor 4E (eIF-4E) binding protein 1]. Phosphorylation of S6K1 and 4E-BP1 by TOR leads to a higher rate of protein synthesis.[1] The Arabidopsis genome encodes two S6 kinase homologs, S6K1 and S6K2, and an in vivo assay showed that RAPTOR1B is interacting with S6K1. Furthermore, S6K1 activity is reduced upon application of osmotic stress and Arabidopsis plants overexpressing *S6K1* are overly sensitive to osmotic stress, a process that is strongly influenced by the TOR pathway.[27]

In yeast and mammals, protein phosphatase 2A (PP2A) is a regulator of cell growth in coordination with nutrient availability and environmental conditions. TAP42 is a regulatory subunit of PP2A and a downstream effector of TOR. Recently, it could be shown that the Arabidopsis TAP42 homolog TAP46 has similar functions as TAP42, positively affects cell growth and can be phosphorylated in vitro by TOR. This suggests that PP2A is a downstream target of TOR signaling via TAP46 and provides evidence for a direct interaction of the two proteins.[32]

Effect of the TOR Pathway on Cell Wall Development

Plants are surrounded by a rigid cell wall that must be able to intermittently enlarge to enable cell expansion. Therefore, TOR signaling is likely to be involved in the coordination of this aspect of cell growth. Indeed, work in yeast suggests a role of the TOR pathway in cell wall integrity signaling.[33] Studies on the Arabidopsis cell wall formation mutant *rol5* (*repressor of lrx1_5*) provided first evidence for such a function in plants. The *rol5* mutant was discovered as a suppressor of the cell wall formation mutant *lrx1*. LRX1 (LRR-extensin 1) is involved in cell wall formation and the *lrx1* mutant shows a defect in the formation of root hairs.[34,35] While an *lrx1 rol5* double mutant shows a suppressed *lrx1* phenotype, i.e., wild-type like root hair development, the *rol5* single mutant develops shorter root hairs, shorter root epidermal cells and exhibits altered cell wall structures compared with the wild type.[20] The ROL5 protein shows 54% identity and 70% similarity to Ncs6p/Tuc1p of yeast (*Saccharomyces cerevisiae*), subsequently referred to as Ncs6p. Ncs6p-like proteins of different organism share conserved motifs, including a PP-loop domain with ATP pyrophosphatase activity, which are also conserved in ROL5. A Δ*ncs6* mutant is hypersensitive to rapamycin, which suggests a potential function of Ncs6p in TOR signaling.[36-39] A second phenotype of the Δ*ncs6* mutant is the lack of thiolated uridines in the wobble position of a subset of tRNAs. This modification is not crucial for cell viability but for efficient protein translation.[40] A full-length *ROL5* construct was able to complement for these phenotypes of the Δ*ncs6* mutant, demonstrating that ROL5 is functionally similar to Ncs6p. Interestingly, it has been shown recently that an accumulation of tRNAs in the nucleus leads to reduced TORC1 activity and upregulated autophagy in human fibroblasts.[41] This finding suggests a link between TOR signaling and tRNAs abundance. To test if ROL5 is involved in TOR signaling in Arabidopsis, *rol5* and wild-type plants were rendered rapamycin-sensitive by overexpression of the yeast *ScFKBP12*. In Arabidopsis, rapamycin-sensitivity causes reduction in root growth.[19] At a low concentration of rapamycin that does not cause a reduction in root growth in the wild type expressing *ScFKBP12* (1.7 ± 0.01 vs. 1.6 ± 0.03 cm without and with rapamycin, respectively; $p = 0.05$), *rol5* mutants with the very same *ScFKBP12* transgene insertion showed significantly shorter roots (1.1 ± 0.02 vs. 0.8 ± 0.01 cm without and with rapamycin, respectively; $p = 0.05$). This demonstrates that the *rol5* mutation renders plants hypersensitive to

rapamycin and suggests a role of ROL5 in TOR signaling. When wild-type plants expressing *ScFKBP12* were treated with rapamycin, they developed alterations in cell wall structures comparable to those of the *rol5* single mutant.[20] Finally, the *lrx1* root hair phenotype could be suppressed through interfering with TOR signaling by rapamycin. (**Fig. 4**).[20] Together, these findings suggest that TOR signaling has an influence on cell wall formation and that ROL5 is a component in this aspect of the TOR pathway in Arabidopsis.

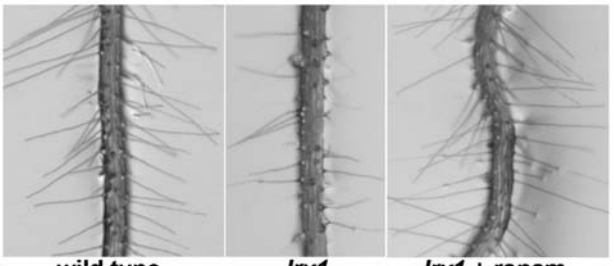

Figure 4. Comparison of Arabidopsis wild-type and *lrx1* mutant plants illustrate the severe root hair formation phenotype in *lrx1*. If *lrx1* plants overexpressing yeast *FKBP12* are treated with rapamycin, the *lrx1* phenotype is suppressed and wild-type like root hairs develop.

Future Perspectives

In animals and yeast, the TOR pathway received tremendous attention due to its importance in regulating cell growth and, consequently, as a potential target for medical applications. In plants, we are only beginning to understand the molecular mechanisms of TOR signaling. While certain proteins are obviously conserved between yeast, animals, and plants, a number of components seem not encoded in the plant genomes analyzed so far. It will be highly interesting to understand the alternative methods developed in plants to relay signals in the TOR pathway. Some of these plant-specific proteins might have functions that are particular to plants such as the development of the cell wall. The molecular-genetic tools available in plants provide an excellent starting point for genetically identifying new components of the TOR pathway that might become valuable means to modify TOR signaling in mammalian cells.

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