Regulation of plant growth and metabolism by the TOR kinase

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Abstract

The TOR (target of rapamycin) kinase is present in nearly all eukaryotic organisms and regulates a wealth of biological processes collectively contributing to cell growth. The genome of the model plant Arabidopsis contains a single TOR gene and two RAPTOR (regulatory associated protein of TOR)/KOG1 (Kontroller of growth 1) and GPI/LST8 (G-protein β-subunit-like/lethal with Sec thirteen 8) genes but, in contrast with other organisms, plants appear to be resistant to rapamycin. Disruption of the RAPTOR1 and TOR genes in Arabidopsis results in an early arrest of embryo development. Plants that overexpress the TOR mRNA accumulate more leaf and root biomass, produce more seeds and are more resistant to stress. Conversely, the down-regulation of TOR by constitutive or inducible RNAi (RNA interference) leads to a reduced organ growth, to an early senescence and to severe transcriptomic and metabolic perturbations, including accumulation of sugars and amino acids. It thus seems that plant growth is correlated to the level of TOR expression. We have also investigated the effect of reduced TOR expression on tissue organization and cell division. We suggest that, like in other eukaryotes, the plant TOR kinase could be one of the main contributors to the link between environmental cues and growth processes.

Specificity of plant development and metabolism

Plant life is profoundly shaped by variations in external conditions. Indeed, unlike animals, plants are autotrophic organisms that can use inorganic nutrient and light as food sources, but cannot move away from unfavourable environments. Therefore, to maintain cellular homeostasis, they have to adapt to a myriad of endogenous and exogenous cues originating from both biotic and abiotic stresses. Indeed plants have to cope with shortage of water, mineral nutrients in the soil and light by expressing and co-ordinating genetic programmes, which allow them to redirect energy resources and re-mobilize nutrients to specific metabolic processes or even organs. For instance, nitrogen starvation stimulates the production of new roots to forage N-rich soil patches, whereas phosphate deficiencies result in the appearance of new specialized roots capable of taking up this nutrient [1]. The rate of growth and development also has to be tightly adjusted to the availability of nutrients. Therefore growth and primary metabolism are highly connected in plants [2].

Whereas in animals the body plan is strictly determined during embryogenesis or larval development, plants have a plastic and often undetermined growth pattern and start as simple organisms that develop new organs throughout their life. New roots, leaves and flowers all originate from unique structures called meristems that contain pluripotent stem cells [3]. The very flexible plant growth pattern allows them to adjust their development to variations in nutrition, light or temperature and implies a continuous production of new organs and a concomitant loss of older parts such as leaves through senescence and death. Therefore the way plants develop new organs during post-embryonic growth is quite different from animals, where most organs are determined during embryonic or larval life. Plant nutritional signalling is also specific since they have to cope with periods of carbon heterotrophy (in the dark or during germination) and autotrophy (in the light). Similarly, heterotrophic (sink, roots or new leaves) and autotrophic (source leaves) organs co-exist in the same organism. Furthermore, after a short period of heterotrophic growth supported by the use of starch or lipids and storage proteins deposited in seed endosperm or embryonic cotyledons, parts of the young seedlings switch to (photo)autotrophic growth. These profound metabolic changes depend on light signalling [4]. As in all other eukaryotic organisms, glucose plays a role as a central regulatory molecule in plants but, unlike heterotrophic cells, accumulation of glucose in actively photosynthesizing plant cells represses carbon accumulation, switching down many metabolic pathways [4].

Notwithstanding these profound differences in metabolism and growth mechanisms, plants such as Arabidopsis can also serve as model organisms for elucidating basic animal
Phylogenetic tree showing the relationships between TOR protein sequences from photosynthetic organisms, yeast, human, *Drosophila* and *Caenorhabditis elegans*

Multiple sequence alignments were first performed on matching protein sequences and the resulting phylogenetic tree was obtained using Neighbour-joining methods and then by defining maximum likelihood (tree obtained from the Phylome database, http://phylomedb.org [31]).

![Phylogenetic tree](image)

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The TOR (target of rapamycin) signalling pathway (see [6,7] for reviews). TOR is a large (approx. 280 kDa) protein kinase that is structurally and functionally conserved. In yeast and animal cells, the TOR kinase is engaged in at least two large protein complexes (molecular mass of approx. 2 MDa), which control a variety of processes contributing to cell growth. These complexes are probably central and conserved components of the eukaryotic cell linking the perception of nutrient availability and of favourable external conditions to cell anabolic metabolism, growth and division by recruiting and regulating the diverse TOR kinase substrates.

**Plant TOR pathway**

The TOR kinase has been found in nearly all eukaryotic genomes, except in some intracellular pathogens belonging to microsporidian species [8]. Photosynthetic organisms such as the model plant *Arabidopsis* all contain at least one TOR gene (Figure 1). The *Arabidopsis* TOR protein sequence (At1g50030.1) is relatively well conserved with the sequence from yeast and animals (approx. 40% of sequence identity; Figure 1). The kinase and FATC domains are well conserved (approx. 70% identity), whereas the FRB [FKBP12 (FK506-binding protein 12)-rapamycin-binding] domain is less conserved. The TOR gene appeared to be expressed in all *Arabidopsis* tissues, whereas the TOR protein was mainly found in young growing tissues such as root tips or emerging leaves, suggesting a post-transcriptional regulation of TOR expression.

Surprisingly, the growth of most land plants, from mosses to angiosperms such as *Arabidopsis*, seems to be unaffected by the presence of rapamycin, even at high concentrations [9]. However, the unicellular green alga *Chlamydomonas reinhardtii* is highly sensitive to rapamycin [9,10]. The lack of rapamycin effects in land plants could be due to the inability of plant FKBP12 homologues to bind rapamycin efficiently [11], since the TOR FRB domain was shown to be still capable of engaging in a ternary complex with rapamycin and either yeast [9] or human [12] FKBP12 protein. Therefore the plant TOR is clearly a rapamycin target. Accordingly it was shown that the expression of the yeast FKBP12 protein in *Arabidopsis* led to rapamycin sensitivity of root growth or polysome accumulation [13]. The molecular mechanisms by which the yeast FKBP12–rapamycin complex interferes with TOR signalling in plants remains to be elucidated.
The TOR kinase operates in at least two high molecular mass complexes named TORC1 and TORC2. Apart from the TOR genes, plant genomes contain genes coding for components of TORC1 such as RAPTOR (regulatory associated protein of TOR)/KOG1 (Kontroller of growth 1) and GβL/LST8 (G-protein β-subunit-like/lethal with Sec thirteen 8) [6]. An interaction between Arabidopsis RAPTOR and the TOR HEAT (huntingtin, elongation factor 3, the PR65/A subunit of protein phosphatase 2A and TOR) repeats was reported [12] and in Chlamydomonas, the LST8 protein was shown to interact with the TOR kinase domain [14]. Conversely, there is so far no evidence for the existence of a plant TORC2 complex since specific components of this complex, such as AVO1/hSIN1 or AVO3/RICTOR, seem to be absent from the genomes of photosynthetic organisms. Nevertheless, these proteins present a low degree of identity, which could make their identification in plants more difficult.

Many homologues of putative TOR regulators and substrates are detected in plant genomes. This includes TCTP (translationally controlled tumour protein [15]), RAG (Ras-related GTP-binding) proteins, P13K (phosphoinositide 3-kinase), AMPK (AMP-activated protein kinase), LKB1 and PDK1 (phosphoinositide-dependent kinase 1). Conversely, there is so far no proof for the presence of TSC (tuberous sclerosis complex), Akt kinase or Rheb proteins (see [16] for a review). Concerning substrates, the Arabidopsis genome contains two S6K (S6 kinase) genes and two genes coding respectively for the PP2A phosphatase-interacting proteins TAP42 (type 2A phosphatase-associated protein 42; TAP46 in plants [17]) and TIP41 (TAP42-interacting protein 41). The S6K domain is highly conserved between plants and animals and the regulatory phosphorylation sites identified in human S6K are also present in the Arabidopsis sequence. Arabidopsis RAPTOR1 interacts with the HEAT repeats of TOR and with S6K1, activity of which is regulated in response to osmotic stress and by plant hormones [12,18]. The phosphorylation of human S6K Thr\(^{389}\) was used as a marker for the TOR kinase activity. This site is conserved in plants and antibodies specific for phosphorylated human Thr\(^{389}\) could therefore be used to follow TOR activity in plants [19].

Role of the plant TOR signalling pathway

In addition to rapamycin resistance, the mutation of the Arabidopsis TOR gene was shown to be embryo lethal [9]. This has probably hampered studies on the plant TOR signalling pathway. Nevertheless, we subsequently isolated Arabidopsis lines overexpressing the TOR gene as a consequence of insertion of T-DNA (transferred DNA) carrying strong promotors in the gene 5′-untranslated region [20]. In parallel, the ectopic expression of an RNAi (RNA interference) construct targeting the TOR FRB domain allowed the recovery of few independent Arabidopsis lines with a reduced TOR expression. In both cases, the level of TOR expression was found to be well correlated with the size of the plants and with resistance to osmotic or salt stress [20]. This represents one of the few studies investigating the impact of variations in TOR expression on the overall size of a multicellular organism. It was also recently reported that inactivation of TOR expression by constitutive RNAi activated autophagic processes, as described in other eukaryotes [21]. Furthermore, the use of conditional silencing of the Arabidopsis TOR gene led to much more severe phenotype with a halt in plant leaf and root growth (Figure 2) accompanied by yellowing of leaves linked to chlorophyll breakdown and premature senescence [20]. These plants also accumulated very high amounts of soluble sugars, amino acids and starch while showing induction of genes such as glutamine synthetase and glutamate dehydrogenase involved in leaf senescence and nutrient remobilization. This suggests that TOR activity is needed to restrain senescence and nutrient recycling and that the TOR signalling pathway could play a central regulatory role in the control of these processes. Arabidopsis plants silenced for TOR expression also displayed a significant reduction in polysome abundance [20]. Inducible TOR RNAi lines were then subjected to transcriptome analysis.

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**Figure 2 | Effect on root and shoot growth of the Arabidopsis TOR gene inactivation by ethanol-inducible RNAi**

Arabidopsis plantlets were first sown in vitro without ethanol, subsequently transferred to vertical agar plates and grown for 10 days with increasing ethanol concentrations.
A strong induction of genes involved in stress responses was observed. Conversely, genes that were repressed upon TOR inactivation were related to the modification of the cell wall and to the signalling pathways of plant hormones like auxin and gibberellic acid. Interestingly, reduction in the TOR kinase activity was recently linked to modifications in the Arabidopsis root hair cell wall [22]. Indeed, a screen for suppressors of the Arabidopsis lrx1 mutation affecting the development of root hairs identified the Rol5 gene that is homologous to yeast NCS6. This gene has been shown to be involved in uridine thiolation of some tRNAs and its mutation confers rapamycin hypersensitivity in yeast [23]. Accordingly, rapamycin treatment of Arabidopsis plants expressing the yeast FKBP12 protein also reverted the lrx1 phenotype. Therefore both rol5 mutations and reduction in TOR activity similarly impacted on cell wall composition and on plant responses to oxidative stress [22].

Mutations of the most expressed RAPTOR gene in Arabidopsis (At3g08850) resulted in early embryonic growth arrest or in slow growth depending on the culture conditions [24,25]. RAPTOR was shown to be phosphorylated by an AMPK activity in animal cells on two conserved serine residues [26]. One of them, Ser792, is conserved in the Arabidopsis RAPTOR protein sequence. It remains to be determined whether this serine residue is also a target of the plant homologues of AMPK (called SnrKs, Snf1-related kinases), which are known to mediate a large part of stress responses in Arabidopsis [27].

As stated above, several well-documented TOR substrates such as the ribosomal S6K, the repressor of RNA polymerase III transcription MAF1 or the TAP42/TAP46 proteins exist in higher plants. It was shown that the overexpression of lily S6K1 resulted in male sterility in transformed Arabidopsis [28]. Moreover, a decrease in Arabidopsis S6K expression led to an increase in ploidy, phenotypic instability and alterations in fertility. It was also found that S6K1 associates with the RBR1 (retinoblastoma-related 1)-E2FB regulating E2F-dependent expression of cell-cycle genes [29].

The TOR signalling pathway also seems to be important for interactions between plants and pathogenic fungi. Indeed, in the vascular wilt fungus Fusarium oxysporum, inactivation of the TOR kinase by nitrogen deficiency appears to be linked with virulence and plant infection [30].

Conclusions

The existence of a TOR signalling pathway in all eukaryotic photosynthetic organisms, from unicellular green algae to land plants is now indubitable (Figure 3). Whereas the occurrence of a TORC2 complex in plants remains to be determined, the presence of a conserved TORC1 complex, with the TOR, RAPTOR and LST8 proteins, has been clearly proven in both algae and higher plants such as Arabidopsis. This TOR complex has important roles in regulating growth, mRNA translation, autophagy, cell wall formation and metabolism but it can be expected that most of the biological outputs, substrates and regulators of the plant TOR signalling pathway are still to be discovered.

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