Sugar metabolism and the plant target of rapamycin kinase: a sweet operaTOR?

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In eukaryotes, the ubiquitous TOR (target of rapamycin) kinase complexes have emerged as central regulators of cell growth and metabolism. The plant TOR complex 1 (TORC1), that contains evolutionary conserved protein partners, has been shown to be implicated in various aspects of C metabolism. Indeed Arabidopsis lines affected in the expression of TORC1 components show profound perturbations in the metabolism of several sugars, including sucrose, starch, and raffinose. Metabolome profiling experiments coupled to transcriptomic analyses of lines affected in TORC1 expression also reveal a wider deregulation of primary metabolism. Moreover recent data suggest that the kinase activity of TORC1, which controls biological outputs like mRNA translation or autophagy, is directly regulated by soluble sugars.

Keywords: target of rapamycin, starch, raffinose, myo-inositol-1-phosphate synthase, TOR serine-threonine kinases

INTRODUCTION

The adjustment of primary metabolism to environmental conditions and to the availability of energy and nutrients is of primary importance to maintain cell homeostasis. Plants, like other eukaryotic organisms, have evolved to make an optimal use of nutrients and to adapt to nutritional deficiencies. This implies that plants have the ability to monitor the amount of available nutrients and energy and to adapt their transcriptional, translational, and metabolic responses to this information. In animals, in which cells are continuously maintained in a rather buffered and uniform supply of nutrients, this regulation of metabolic activity and cell growth at the cellular level is mainly driven by growth factors and hormones. For plants, nutrients provide not only the food for growth but also the signals for growth. Indeed nutrients serve both as the resources by which the cell increases mass and generates energy and as the signals controlling the metabolic and developmental programs which optimize survival under particular nutritional states.

Furthermore, plants experience rapid, sudden, and often long changes from optimal growth conditions and they must be able to both monitor precisely these changes and to trigger counter-measures ensuring survival and adaptation while maintaining growth and biomass production. In plants, like in other eukaryotes, the signaling pathway involving the TOR (target of rapamycin) protein kinase has emerged as an evolutionary conserved and critical link between external cues and metabolic and growth adaptations (see Wullschleger et al., 2006; Ma and Blenis, 2009; Loeuillot and Hall, 2011; Laplante and Sabatini, 2012; Corre et al., 2013 for general reviews and Dobrenel et al., 2011; John et al., 2011; Robaglia et al., 2012 for reviews on the plant TOR signaling pathway).

Target of rapamycin was identified 20 years ago in yeast in a screen for mutations conferring resistance to rapamycin, an antibiotic that stops growth and induces a shift to the G0 quiescent stage (Hetman et al., 1991). It was later shown that rapamycin inhibits TOR by triggering the formation of an artificial complex between the FRB (FKBP12-rapamycin binding) domain and the small FKBP12 protein (Wullschleger et al., 2006). Rapamycin treatment inhibits some of the TOR-linked activities and results, in yeast and animal cells, in the accumulation of the storage compound glycogen, in translation decrease and in the induction of autophagy (Schmelzle et al., 2004; Rohde et al., 2008; Broach, 2012; Corre et al., 2013). These changes also occur in nutrient-starved cells (Rohde et al., 2008; Broach, 2012), which suggests that TOR is one of the main components of the transcription chain linking nutrient signaling to cellular adaptations. Indeed a wealth of studies, both in yeast and in animals, have clearly established that the TOR kinase is activated by external signals like the availability of amino acids or the presence of hormones, and then controls a myriad of biological outputs including transcription of mRNA, translation, ribosome biogenesis, translocation of regulatory proteins, autophagy, and storage of reserve compounds (see above reviews). This review will mainly focus on the cross-talk between the conserved plant TOR kinase signaling pathway and C metabolism with a particular emphasis on C storage compounds.

THE TOR KINASE

The large TOR kinase associates in high molecular mass complexes with other conserved protein partners (Wullschleger et al., 2006; Loeuillot and Hall, 2011; Wang and Proud, 2011). In yeast and animals, the TOR kinase functions in two distinct multiprotein complexes named TOR complex 1 (TORC1) and TOR complex 2.
Accordingly no interactions were detected between Arabidopsis Xiong and Sheen, 2012) and were presenting signs of constitutive Arabidopsis also found in plants (Menand et al., 2002; Anderson et al., 2005; proteins (TOR, KOG1/RAPTOR, and LST8/G betaL), which are the TORC2 complex contains LST8/G betaL with specific proteins like ATG5/ATG10/BetaTOR and ATG5/SN1 (Walschugler et al., 2006). The existence of the TORC2 complex in plants has not been proven so far but it may represent a more recent addition to the TOR signaling pathway.

It was previously thought that rapamycin, even at important doses, does not affect plant growth. Indeed plant FKBP12 proteins carry mutations that would preclude the formation of a TOR-rapamycin-FKBP12 ternary complex (Xu et al., 1998; Menand et al., 2002; Mahfouz et al., 2006; Sormani et al., 2007). Accordingly no interactions were detected between Arabidopsis TOR and FKBP12 proteins using two-hybrid techniques (Mahfouz et al., 2006; Sormani et al., 2007). However, the Arabidopsis TOR FRB domain could bind the yeast (Sormani et al., 2007) or human (Mahfouz et al., 2006) FKBP12 proteins in the presence of rapamycin. This opened the possibility of increasing plant sensitivity toward rapamycin by expressing the yeast FKBP12 protein (Sormani et al., 2007; Leiber et al., 2010; Ren et al., 2012).

Conversely, the unicellular green algae Chlamydomonas is sensitive to moderate levels of rapamycin (100–500 nM), a concentration range similar to the one necessary to inhibit yeast growth (Heitman et al., 1991; Crespo et al., 2005). This can be explained by the fact that the algal FKBP12 protein is closer to human or yeast homologs and the residues critical for binding rapamycin are conserved only in Chlamydomonas. Nevertheless, it was recently shown that rapamycin, when added repeatedly at high concentrations (1–10 μM) to liquid cultures of Arabidopsis, could affect plant growth and development (Xiong and Sheen, 2012).

This varying and reduced susceptibility of plants to rapamycin has clearly delayed the development of molecular studies on the plant TOR signaling pathway. Moreover the disruption of the AtTOR gene by T-DNA insertions was shown to be embryonic lethal (Menand et al., 2002; Ren et al., 2011), which precluded the use of these mutants to further study the role of TOR in plants. To circumvent these difficulties we have produced constitutive and conditional (amiRNA) lines which allow a stable or conditional silencing of the AtTOR gene (Deprost et al., 2007). This study and other reports using estradiol-inducible artificial microRNA (amiRNA) showed that, when the expression of AtTOR was silenced, plant growth was arrested and several metabolites accumulation in animal muscles and yeast (Schmelzle et al., 2004; Cornu et al., 2013). Conversely TOR inhibition in the liver decreases the level of stored glycogen and the animals become hyperglycemic, a situation also found in type 2 diabetes. This suggests a prominent role of TOR in maintaining animal glucose homeostasis (Cornu et al., 2013). In yeast TORC1 inhibition by rapamycin triggers a switch from fermentation to respiration by reducing the expression of genes encoding glycolytic enzymes.
Van den Ende, 2011). The levels of raffinose, of its precursor oligosaccharides (RFOs, precursors like UDP-glucuronate and of the raffinose family signaling lipids (phosphatidyl)inositol-phosphate (PIP), of cell wall a central C-metabolite that serves in the synthesis of the sig- naling molecule myo-inositol-1-phosphate, which in turn activates glycolysis and raffinose synthesis in response to stresses. Conversely TORC1 represses the synthesis of reserve molecules. Animal reserve compounds are in red, plant ones are in green. Activation is shown by arrows. B) Accumulation of starch following inactivation of the TORC1 complex in percentage of the control wild-type (WT). The effect of the inactivation of the TORC1 component LST8 on starch accumulation was investigated in insertion mutants. Results from the analysis of stl8 mutants are from Moreau et al. (2012) and are compared to the corresponding control WT (Col8). A time-course experiment shows the accumulation of starch following inactivation of TOR by estradiol-inducible RNAi (24–72 hours) after ethanol induction. Deprost et al. (2007). The B-leaf (18) point is from Caldana et al. (2013) using estradiol-inducible amiRNA lines.

Clement, personal communication; Sulpcie et al., 2010). A survey of metabolite profiling experiments shows that raffinose accumulates in stress situations like high light, nitrogen starvation, or high salt (G. Clement, personal communication and unpublished data).

A common trend that emerges from the analysis of stressed Arabidopsis plants affected in the activity of the TORC1 complex is the decrease in raffinose and galactinol accumulation. Ren et al. (2012) observed lower levels of starch and raffinose contents are negatively correlated with the accumulation of high amounts of soluble sugars, amino acids, and starch (Figure 1B). This suggests that TOR activity is needed to restrain senescence and could thus be involved in the regulation of life span in Arabidopsis (Deprost et al., 2007; Ren et al., 2012). A recent study also showed that TOR inhibition in Arabidopsis by inducible amiRNA results in high levels of starch accumulation (Figure 1B) together with increased levels of TAG (Caldana et al., 2013). A concomitant increase in TCA cycle intermediates was also detected after TOR inhibition by either amiRNA (Caldana et al., 2013), the treatment by rapamycin of Arabidopsis lines expressing a FKBP12 protein (Ren et al., 2012) or in stl8 mutants (Moreau et al., 2012). The same perturbation in the TCA cycle was observed in yeast treated with rapamycin (De Virgilio and Loewith, 2006).

In the study by Sulpcie et al. (2009) described above, it was found that rapamycin biomass negatively correlated with the amount of starch but a strong positive correlation was detected with the expression of myo-inositol-1-phosphate synthase 1 (MIPS1/At4g39800). MIPS is conserved in all eukaryotes and catalyzes the first committed step in the synthesis of myo-inositol, a central C-metabolite that serves in the synthesis of the signaling lipid (phosphatidylinositol-phosphate (PIP), of cell wall precursors like UDP-glucuronic acid and of the raffinose family oligosaccharides (RFOs, Figure 2; for a review see Valluru and Vain de Eede, 2011). The levels of raffinose, of its precursor galactinol and of myo-inositol, which serves as a cofactor in this biosynthetic pathway, are often strongly correlated (Figure 2; G.
It is now clear that inhibiting the TORC1 activity results in starch and TAG accumulation (Dobrenel et al., 2011; Caldana et al., 2013), a decrease in biomass production but also a decrease in protein concentration and mRNA translation (Deprost et al., 2008; Sulpice et al., 2009, 2010). The signals triggering starch accumulation and re-routing of C fluxes in response to TORC1 inactivation may contribute to the close link between starch, protein, and biomass observed in plants (Sulpice et al., 2009, 2010). The MIPS genes could therefore serve as a hub for adjusting the plant metabolism to changes in environmental conditions. The inducible overexpression of the bZIP11 transcription factor, which is normally up-regulated by the SnRK1 kinase, results in an augmented level of raffinose (Ma et al., 2012). This is consistent with the fact that raffinose is also accumulated in response to multiple stresses (Valluru and Van den Ende, 2011). Indeed this could possibly be the result of the activation of the SnRK1 kinase in stress conditions (Robaglia et al., 2012). Nevertheless it is surprising, given the expected opposite role of the TOR and SnRK1 kinases (Robaglia et al., 2012), that TOR also seems to be required for raffinose production. One explanation could be that TORC1 activity is also needed for SGF11 expression and it would be interesting to determine if the bZIP11-induced accumulation of raffinose is TOR-dependent.

CONCLUSION

It is now clear that inhibiting the TORC1 activity results in starch and TAG accumulation (Dobrenel et al., 2011; Caldana et al., 2013), a decrease in biomass production but also a decrease in protein concentration and mRNA translation (Deprost et al., 2007; Sormani et al., 2007; Ren et al., 2012; Xiong and Sheen, 2012; Caldana et al., 2013). Nevertheless it is surprising, given the expected opposite role of the TOR and SnRK1 kinases (Robaglia et al., 2012), that TOR also seems to be required for raffinose production. One explanation could be that TORC1 activity is also needed for SGF11 expression and it would be interesting to determine if the bZIP11-induced accumulation of raffinose is TOR-dependent.
Since the inactivation of TOR results, as in other eukaryotes, in the accumulation of reserve molecules in plants (TAG and starch), it can be anticipated that the regulation of TOR activity in developing seeds may also be of importance for the synthesis of seed storage compounds. Moreover, using TOR inactivation to redirect fluxes toward reserves compounds like starch, which are easier to store than lipopolymers, could foster the use of plants for the production of biofuels and other bio-based components.

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