

REGULATION OF PLANT GROWTH BY THE TARGET OF RAPAMYCIN (TOR) PATHWAY

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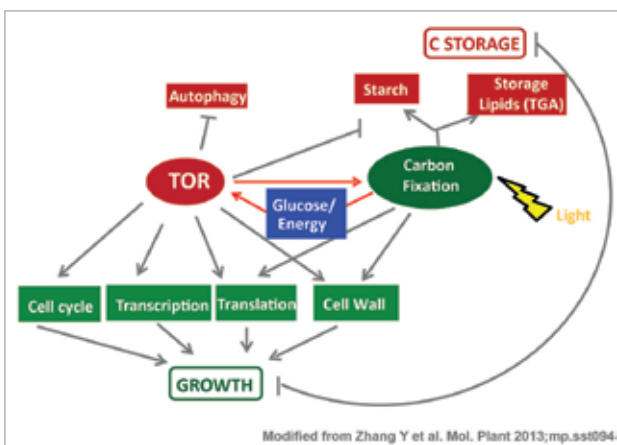


Figure 1. TOR controls several biological processes including transcription, translation and central energy metabolism, which collectively contribute to growth

Rising demand for food and fuels makes crucial to develop breeding strategies for increasing crop yield/biomass. Plant biomass production is closely linked to growth and depends on a tight regulation of a complex signalling network that integrates external and internal factors. One of the key regulators of growth in all eukaryotes is the Target of Rapamycin (TOR) kinase pathway. TOR integrates environmental cues such as energy status or nutrient availability into cell growth and proliferation. In the model plant *Arabidopsis thaliana* the levels of TOR expression are positively correlated with growth. However little is known about the mode of action of TOR controlling growth in photosynthetic organisms. Over the last years, the main goal of our group has been to understand the molecular mechanisms involved in growth and metabolism of plants modulated by TOR.

Recently, experimental evidences revealed that the disruption of this essential protein leads to severe growth phenotypes, which are based on the essential reprogramming of the plant metabolism. The identification of plant TOR as a potent metabolic switch offers a novel route for biotechnological optimization of plant carbon partition for bioenergy production. However, there are still many gaps about how TOR controls those metabolic decisions and regulates growth, especially in C_4 plant species used for biofuel production due to their capability to produce biomass at faster rates. Therefore, our aim is to use a systems-oriented approach to dissect TOR signalling network involved in plant growth and metabolism using both C_3 and C_4 model species (*Arabidopsis* and *Setaria* ssp. respectively). Such approach is expected to reveal how plants adjust their metabolism ratios in order to simplify the conversion of plant biomass into biofuels. Moreover, the comparison between these two photosynthetic systems will allow further comprehension of the growth regulatory network mediated by TOR contributing to the efforts in bioengineering C_4 features in C_3 crops. From a technological standpoint, the proposal includes the establishment of a metabolomic oriented lab that will serve as facility unit for the community and also provide the centerpiece for human resources training.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We first developed a gas chromatography coupled to a mass spectrometry (GC-MS) platform for profiling primary metabolites. This platform allowed us to create a database of polar metabolites from several plant species, including *Arabidopsis thaliana*, *Setaria italica*, *Setaria viridis*, and sugarcane.

In order to investigate the relationships between environmental factors and TOR signaling pathway in carbon allocation, we are performing several analyses in a comparative manner, using model plants with different mechanisms of photosynthesis: *Arabidopsis thaliana* (C₃) and *Setaria viridis* (C₄). To quantitatively and kinetically control the TOR levels in both species in a similar way, seedlings were grown hydroponically for about 12 days prior to treatment with the ATP-competitive TOR kinase inhibitor AZD-8055 (TOR repressor) or DMSO (control). Our data suggested a clear ceased growth phenotype of *Setaria* and *Arabidopsis* after 2 days of drug treatment. Biochemical and metabolomics analysis of *Setaria* and *Arabidopsis* seedlings treated with AZD revealed differences in carbon allocation mechanism mediated by TOR. Whereas *Arabidopsis* seedlings treated with AZD presented a starch excess phenotype (elevated rates of starch synthesis independently from the time of drug administration), no differences in starch content were observed in *Setaria* seedlings. In order to validate these data, we also generated inducible artificial micro RNA transgenic lines targeting the TOR in *S. viridis*.

We are currently working on two hypotheses about TOR mode of action i) directly phosphorylation enzymes of starch synthesis such as SPS and ii) regulation of other proteins (e.g., kinases) that can modulate the activity of enzymes involved in starch metabolism (e.g., SnRK1). These preliminary results will be useful as starting point to characterize the mode of action of TOR on starch metabolism and to dissect its function in plants.

MAIN PUBLICATIONS

Jorge TF, Rodrigues JA, Caldana C, Schmidt R, van Dongen JT, Thomas-Oates J, Antonio C. Mass spectrometry-based plant metabolomics: metabolite responses to abiotic stress mass spectrometry reviews. doi:10.1002/mas.21449. [Epub ahead of print]

Abdelnur PV, Caldana C, Martins MCM. 2014. Metabolomics applied to bioenergy. *Chemical and Biological Technologies in Agriculture*. 1: 22.

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