University of Fribourg / Faculty of Science / Department of Biology

Homeostatic control and isolation of new targets of TORC1 in budding yeast

Serena Raucci

The target of rapamycin complex 1 (TORC1), a conserved serine/threonine kinase complex, is the central cell growth controller in all eukaryotic organisms. Localized at the vacuolar membrane in yeast and, when active, at the lysosomal surface in mammals, it modulates multiple catabolic and anabolic processes by phosphorylating its targets in response to several stimuli such as amino acids and different kinds of stresses. The regulation of TORC1 by amino acids occurs via the heterodimeric yeast Rag GTPases Gtr1-Gtr2 (RagA/B-RagC/D in mammals) and their regulators that include GTP exchange factors (GEFs) and GTPase activating proteins (GAPs). We previously reported that in yeast, the Lst4-Lst7 complex functions as a GAP for Gtr2. This complex localizes at the vacuolar surface upon amino acid starvation and is released from the vacuole into the cytosol when cells are re-fed with nutrient.

In the first chapter of this thesis, we present a model in which the dynamic localization of the Lst4-Lst7 complex at the vacuole is regulated by TORC1-dependent phosphorylation of Lst4. We demonstrate that TORC1 directly phosphorylates Lst4 on several residues (*in vivo* and *in vitro*) within the intra-DENN loop, an unstructured region in the DENN domain of Lst4. Interestingly, the intra-DENN loop is necessary and sufficient to anchor the Lst4-Lst7 complex at the vacuolar membrane. While the Lst4 phosphorylation status regulates the localization of Lst4-Lst7 complex it does not affect its GAP activity towards Gtr2. Notably, the expression of an Lst4 allele that cannot be phosphorylated by TORC1 induces hyperactivation of TORC1, leading to growth defects when cells are grown on a poor nitrogen source. In sum, our data reveal a feedback mechanism that prevents TORC1 hyperactivation in the presence of amino acids thereby contributing to the regulation of the homeostasis of the TORC1 activity.

In the second chapter, we elaborate a new method for the identification of unknown TORC1 targets. We combine the TORC1 *in vitro* kinase assay with mass spectrometry analysis using yeast-purified proteins as substrates of TORC1. As a proof of principle, we focus on Atg proteins which allowed us to discover new targets of TORC1. Among these, we identified Atg29, which is also phosphorylated by TORC1 *in vitro* when purified from bacteria.

In the third chapter, we analyze the effects of the plant hormone Indole-3-acetic acid (IAA) on yeast cell growth, providing evidence *in vivo* and *in vitro* that this compound is a direct inhibitor of TORC1. We also present a screening of the yeast knockout collection performed in the presence of IAA or rapamycin, in which we compare the mutants hypersensitive to IAA with those hypersensitive to rapamycin. Combined, our data suggest that the TORC1 pathway is involved in the response to IAA, though this compound also impinges on other pathways important for growth.

In the final chapter, we discuss about open questions and future perspectives that could follow the studies addressed in the previous chapters.

Jury:
Prof. Claudio De Virgilio (Thesis supervisor)
Prof. Robbie Loewith (exteranl co-examiner)
Prof. Jörn Dengjel (internal co-examiner)
Prof. Urs Albrecht (President of the jury)