# New insights into the role of the TORC1 subunit Tco89

Hélène Blöchlinger

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Known as a master regulator, TORC1 promotes and regulates cell growth. It remains elusive, how TORC1 binds to the heterodimeric Gtr1-Gtr2 Rag GTPases by which it is regulated. The expression of a nucleotide-free variant of Gtr1 exhibits a strong growth defect in *gtr1Δ* cells that can be suppressed by loss of the TORC1 subunit Tco89. This suggests that the Rag GTPases can, in addition to their known activating role, also inhibit TORC1 and that the subunit Tco89 may be involved in mediating this effect. The aim of this master thesis was to characterize Tco89 regarding its potential regulation of TORC1 activity.

In order to pinpoint functional amino acids in Tco89 that contribute to TORC1 regulation, we established a screen to select for Tco89 mutant variants, that suppress the growth defect in *gtr1Δ* cells expressing $Gtr1^{S20L}$. The screen was set up by using both a genomic- and a plasmid-based approach. In addition, we genomically fused a metabolic marker (*i.e. HIS3* coding for imidazoleglycerol-phosphate dehydratase) to the *TCO89* open reading frame, which allowed us to eliminate in the respective selection those suppressor mutations that caused a frameshift or stop codon. Accordingly, we were able to confirm that the enzymatic activity of His3 fused to Tco89 is sufficiently functional to render otherwise *his3*Δ auxotrophic cells histidine prototroph if the fusion construct was expressed from a constitutive promoter.

In parallel to this unbiased genetic screen, we also studied a Tco89 mutant allele, that is substituted with the non-phosphorylatable amino acid alanine at 23 potential TORC1 target residues. Our data give a first insight into the role of posttranslational modifications on Tco89 and suggest that the phosphorylation of Tco89 contributes to normal TORC1 activity.

Prof. Dr. Claudio de Virgilio